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THE UNIVERSITY OF ALBERTA

# Basic and Clinical Pharmacokinetics of Ketoprofen Enantiomers

by

Robert Thomas Foster

(C)

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN

PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

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IN

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Date: July 20, 1988

To my wife,

Caroline,

who has given me love and support

throughout my studies.

## ABSTRACT

The treatment of osteo- and rheumatoid arthritis very often requires medicinal and/or surgical intervention. The 2-arylpropionic acid type (2-APA) non-steroidal antiinflammatory drugs (NSAIDs) contribute significantly to the arsenal of pharmacologic interventions available. An important compound within this class of 2-APA derivatives is ketoprofen (KT), marketed for some years in Europe and now available for use in Canada. To date, studies of KT pharmacokinetics in man and animal have dealt with total (R(-) plus S(+) enantiomer) concentrations, despite the fact that KT is a chiral compound and is administered as the racemic mixture. Furthermore, pharmacologic activity resides primarily in the S(+)-enantiomer; the R(-)-enantiomer has little or no activity. In this work, ketoprofen was studied 1) in healthy subjects, 2) in young and elderly arthritic patients, 3) in patients requiring aspiration of synovial fluid from the knee, 4) in patients following cholecystectomy surgery, in the presence and absence of probenecid and 5) in the Sprague-Dawley rat. These studies were conducted following the developement of a stereospecific, sensitive and convenient high performance liquid chromatographic (HPLC) assay. In man, there was little or no stereoselectivity in the pharmacokinetics of intact KT in plasma. Although there was virtually no conjugated drug in the plasma of young subjects and young patients, there was a substantial concentration of glucuroconjugated KT in elderly patients' plasma

with greater S than R configuration. In urine, the drug was eliminated entirely as the conjugate; S-KT was predominant. Urinary elimination of these conjugates in elderly patients was reduced as a function of age. Ketoprofen was eliminated to a minor extent through bile. The biliary pathway did not completely compensate for reduced urinary excretion of KT caused by renal dysfunction or probenecid. Concentrations of the two enantiomers in synovial fluid did not appear to be different, however maximal concentrations of both isomers in the joint were attained at a later time than in plasma. Man did not appear to invert R(-)- to S(+)- KT. In rat, substantial stereoselectivity in the disposition of KT enantiomers in plasma was observed. Moreover, rat is able to extensively bioinvert R(-)- to S(+)-KT both systemically and presystemically in the gut. The major elimination pathway in rat is through bile, and not urine.

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## GLOSSARY OF ABBREVIATIONS

| | |
|---|---|
| ANOVA | Analysis of variance |
| AUC | Area under plasma concentration-time curve |
| $C_{max}$ | Maximum concentration |
| Cl/F | Oral clearance |
| ° | degrees Celcius |
| F | Oral bioavailability |
| h | hour(s) |
| HPLC | High-performance liquid chromatography |
| IS | Internal standard |
| ip | intraperitoneal |
| iv | intravenous |
| kg | kilogram(s) |
| KT | ketoprofen |
| L | liter(s) |
| $LD_{50}$ | lethal dose for 50% |
| uL | microliter(s) |
| um | micrometer(s) |
| mg | milligram(s) |
| mg/L | milligrams per liter |
| min | minute(s) |
| mL | milliliter(s) |
| n | number of observations |

| | |
|---|---|
| nm | nanometer(s) |
| OA | osteoarthritis |
| PB | Probenecid |
| po | oral |
| q6h | every six hours |
| RA | rheumatoid arthritis |
| $r^2$ | correlation |
| s | second(s) |
| SD | Standard deviation |
| $\Sigma Xb$ | Cumulative biliary excretion |
| $\Sigma Xu$ | Cumulative urinary excretion |
| t½ | half-life |
| $T_{max}$ | Time to peak |
| UV | Ultraviolet |
| Vd | Volume of distribution |

# 1. INTRODUCTION

## 1.1. The Drug

### 1.1.1. History

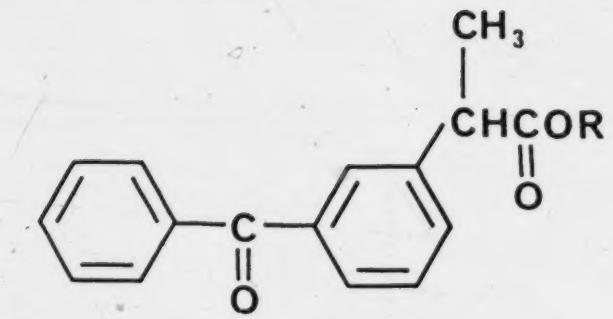
Ketoprofen (KT) is a 2-arylpropionic acid (2-APA) type non-steroidal antiinflammatory drug (NSAID). This compound was first synthesized by Farge, Messer and Moutonnier at Rhone-Poulenc in France, in 1967, following the introduction of ibuprofen, a prototype, 3 years earlier. Later, in 1973, KT was introduced as an antiinflammatory drug onto the European market. Today, the drug is used extensively in many countries in the treatment of both rheumatoid and osteoarthritis.[1]

Clinical experience with KT is now extensive, exceeding more than 3 million patient-years. Therapeutic experience has rated KT as being at least as effective as aspirin, indomethacin, and ibuprofen in rheumatoid arthritis and similar to aspirin in osteoarthritis.[2]

### 1.1.2. Physicochemical Properties

The chemical structures of KT, (±)2-(3-benzoylphenyl) propionic acid, and its glucuronic acid conjugate are depicted in Figure 1-1.

Ketoprofen is a white, or slightly colored, odorless, sharp-bitter tasting crystalline powder, with a molecular weight of 254.29.[3] The drug is soluble in benzene, ethanol, chloroform, acetone, ether and alkaline solutions, but is practically




R= 1; H

2; $C_6H_9O_6$

Fig. 1-1. Chemical structures of KT (1) and its glucuronic acid conjugated metabolite (2).

insoluble in water, and has a melting point of 93-95°.[3]

Due to the presence of an asymmetric carbon center, KT exists as enantiomers and is marketed as the racemic mixture under numerous trade names.[4] Both enantiomers have been isolated and assigned absolute configurations.[3]

1.1.3. Mechanism of Action

The chief actions of the NSAIDs, including KT, are due to their antiinflammatory, analgesic and antipyretic properties.[2,5-7] The therapeutic activity of NSAIDs, in general, appears to be due primarily to their inhibition of prostaglandin synthesis.[8] More specifically, NSAID activity involves the reversible inhibition of cyclooxygenase, the enzyme responsible for catabolism of arachidonic acid into prostaglandins and thromboxanes.[5,6,9]

More recently, however, the notion that NSAIDs solely inhibit the cyclooxygenase enzyme has been questioned. The lipooxygenase pathway of arachidonic acid metabolism has been implicated in the inflammatory response, as products such as leukotriene $B_4$ are significant inflammatory mediators. Interference with leukotriene synthesis, and thus inflammation, may be attributed to NSAID therapy.[6]

The role of interleukin 1 in the pathogenesis of a number of chronic diseases, including rheumatoid arthritis, is becoming increasingly clear.[10] Despite this knowledge, the effect of NSAIDs on interleukin 1 is not clear. It is known that cyclooxygenase inhibitors interfere with some of the effects of interleukin 1, but the interaction is not specific.[11]

Other pharmacologic properties that KT possesses, and which may be related to antiinflammatory and analgesic activity include its bradykinin inhibitory, and lysosomal membrane stabilizing effects.[2,7]

1.1.4. Side Effects and Toxicity

The results of both short- and long-term toxicology tests on animals and tests on humans indicate that KT is a relatively safe drug.[7] Although the side effects may be numerous, they are usually not severe.[12] The reported side effects include gastrointestinal, renal, hepatic, hematologic, anaphylactoid and skin reactions, and central nervous system and special senses toxicities. These side effects have recently been summarized in an excellent review.[6]

The $LD_{50}$ in many mammalian species has been investigated both in acute and subacute studies.[2] In acute studies, drug toxicity was characterized by sedation, adynamia, diarrhea, and emesis. Gastrointestinal lesions were revealed at autopsy. Subacute studies resulted in gastrointestinal toxicities in the rat and dog, whereas the baboon had little gastrointestinal irritation. Rats also exhibited renal toxicities.[2]

Teratogenicity studies of KT administered to mouse, rat and rabbit were carried out.[7] The drug was devoid of any embryotoxic effects, except for rabbits receiving high doses. In this case, the observed slight embryotoxic effect was attributed to the general decline of the condition of the rabbits.

There was no evidence of carcinogenicity or mutagenicity of KT when standard screening assays were performed.[2]

### 1.1.5. Pharmacokinetics

#### 1.1.5.1. *Absorption.*

Pharmacokinetic studies in humans show that KT is rapidly absorbed after an oral dose[13-16] and that absorption is virtually complete.[13,17] As expected, the rate of absorption is delayed by administration of various sustained release products.[18,19] Although the rate of absorption may also be altered by the presence of food,[16,20] the extent of absorption does not change. Similar conclusions were made when KT and sucralfate were administered concurrently.[14] Both the rate and extent of absorption, however, show circadian changes where bioavailability is maximal at 0700 h.[21]

#### 1.1.5.2. *Distribution*

Ketoprofen is rapidly distributed,[13] with a volume of distribution (Vd) of approximately 0.1 L/kg.[8] The drug is highly protein bound (approximately 99%) mainly to albumin.[22] Ketoprofen slowly penetrates into synovial fluid, and has a mean residence time in the knee joint about three times that of the systemic circulation.[23]

#### 1.1.5.3. *Metabolism and Elimination.*

Ketoprofen is extensively metabolized, forming an ester glucuroconjugate.[2,17,24] The conjugated drug is then eliminated via the kidneys, to the extent of 50-90% of the administered dose.[17,24] Although previous investigators reported up to 50% of KT was eliminated in the urine as intact drug,[17,24] Upton *et*

*al.* have demonstrated that, in fact, virtually 100% of the drug excreted renally is as conjugates.[25] This discrepancy is likely due to the inherent instability of the ester glucuronide conjugates, which quickly hydrolyze back to the parent drug during various storage and handling procedures.[25,26] A hydroxylated metabolite of KT has also been identified in rat[27] although the presence of this metabolite in man has not been proven.

The process of conjugation and subsequent renal elimination has recently been studied in elderly patients, and in patients with impaired renal function. In one study,[28] no observable changes occurred in the elderly, whereas another study[29] suggested that glucuronidation in the elderly was impaired. In patients with compromised renal function, a longer t½ for KT was observed[30] which, according to the authors, was expected as conjugated KT relies on renal function for excretion.

### 1.1.6. Variability in Response

In general, there is a poor correlation between dose and/or concentration of administered NSAIDs and response between individuals.[6] Apart from difficulties in assessing patient response to NSAID therapy,[6] numerous suggestions have been made as to why this might be the case. These suggestions can be broadly categorized to encompass either pharmacodynamic or pharmacokinetic principles.

Pharmacodynamic factors which may be involved in explaining the basis of variability of response to NSAIDs include 1) there are numerous mechanisms of action (see section 1.1.3.), 2) the

contribution of each of the mechanisms of action to the overall effect of the drug is not clearly understood, and 3) there are numerous specific cellular effects which are independent of cyclooxygenase inhibition.[31] Thus, the clinical relevance and contribution of various pharmacodynamic processes is a matter of question.

Although there is a better correlation between concentration (as opposed to dose) of administered NSAID and effect, this correlation has been suprisingly difficult to demonstrate. For drugs such as naproxen[32] and fenclofenac[33] a significant correlation between plasma concentration and effect has previously been reported. For other drugs such as KT, however, no such correlation has been observed. As a result, dosing of many of the NSAIDs including KT is largely empirical.

In addition to the above mentioned pharmacodynamic obstacles, there are numerous pharmacokinetic problems which must be considered when attempting to define a concentration *versus* effect relationship for many of the NSAIDs including KT. Many of these pharmacokinetic obstacles regarding NSAID therapy have recently been reviewed by Day *et al.*[6] and include factors such as binding of drug to plasma and synovial fluid proteins, kinetics of transfer of drug into synovial fluid, terminal elimination t½, metabolism and excretion, and the effects of various pathologies and/or age on drug disposition. However, perhaps one of the most important considerations deals with the stereoselective pharmacokinetics of the 2-APA NSAIDs.[34-37]

### 1.1.7. Rationale

As the 2-APA NSAIDs are chiral in nature, an important aspect of their use in rheumatoid and osteoarthritis is the stereoselectivity of action. The antiinflammatory properties are ascribed mainly to the S-enantiomer (eutomer), whereas the R-enantiomer (distomer) has little or no activity. As the enantiomers differ in their inherent activity, it is therefore clear that attempts to correlate concentrations of total KT (R- plus S-enantiomer) with effect are pointless. Nevertheless, virtually all data to date deal with total, rather than enantiomeric concentrations of drug. To further complicate this issue, the R-enantiomer may be metabolically inverted to the more active S-enantiomer. The general applicability of bioinversion to all members of the 2-APA class of NSAIDs has been suggested.[35] Therefore, depending on the rate of inversion, the R-enantiomer may be considered as either a pro-drug, or as 'isomeric ballast'.[37] Despite the fact that these compounds are marketed and administered as racemic mixtures, little information exists regarding the enantioselective pharmacokinetics in humans. It was therefore proposed that the enantiomers of KT, a 2-APA NSAID, would be studied in detail in an attempt to delineate the pharmacokinetics of both the R- and S-enantiomers in man and rat.

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## 2. HPLC ASSAY OF KETOPROFEN ENANTIOMERS IN HUMAN PLASMA AND URINE*

### 2.1. INTRODUCTION

Ketoprofen (KT), a 2-arylpropionic acid type (2-APA) non-steroidal antiinflammatory drug (NSAID), contains a chiral center, and is marketed as a 50:50 racemic mixture of the S(+)- and the R(-)-enantiomers. The S(+)-isomer is usually responsible for the antiinflammatory activity of the 2-APAs, whereas the R(-)-isomer is less active, or inactive.[1] It is therefore important to measure levels of the active enantiomer when seeking a correlation between drug levels in blood and clinical efficacy and/or toxicity. The resultant information using stereoselective techniques likely forms the basis of a more rational approach to therapy with KT.

Singh *et al.*[2-4] previously described a stereoselective gas chromatographic method for the separation of several NSAID enantiomers, including KT. However, the method was time-consuming, as derivatization included a lengthy heating period. Sallustio *et al.*[5] reported an enantiospecific high performance liquid chromatographic assay for ketoprofen and fenoprofen. This method was also time consuming, and lacked the desired sensitivity for KT. Bjorkman [6] reported an HPLC assay for the enantiomers of

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indoprofen. The method involved extraction of unchanged drug, conversion to a mixed anhydride with ethyl chloroformate, derivatization with L-leucinamide, extraction of the formed diastereoisomers with an organic solvent, evaporation and injection into the HPLC. Using this sample preparation method, Bjorkman noticed interfering peaks in his chromatograms. We adopted the above method to assay KT enantiomers in human plasma and urine and noticed three small interfering peaks. Hence, the assay was substantially modified. After submission of this paper, it was brought to our attention by the Editor of the Journal that a paper by Bjorkman [7] describing an HPLC assay of KT enantiomers in plasma, based on the method used in indoprofen determination, had been recently accepted for publication. To avoid the interfering peaks, Bjorkman [7] used an ion-pairing procedure to extract KT enantiomers from plasma. His assay, however, as compared to the one reported here involved a lengthier sample preparation process and was less sensitive. The method reported herein also has the advantage of being applicable to the analysis of conjugated KT in urine.

## 2.2. EXPERIMENTAL

### 2.2.1. Sample preparation

To 0.5 mL plasma samples containing KT was added 100 uL of an aqueous solution of 100 ug/mL racemic fenoprofen calcium (Eli Lilly, Indianapolis, IN., USA) as internal standard (IS) and 100 uL 0.6 M sulfuric acid as acidifier. The resultant was extracted with a

mixture of 5% isopropanol in isooctane after Vortex mixing for 30 s and centrifuging at 1800 relative centrifugal force for 5 minutes. The organic phase was transferred to a clean glass tube and 3 mL water (HPLC grade, Fisher) was added. Samples were again Vortex mixed for 30 s and centrifuged for 3 minutes. The organic layer was discarded and 200 uL of 0.6 M sulfuric acid was added. Chloroform (3mL) was added to this, and the samples were Vortex mixed (30 s) and centrifuged (3 min). The aqueous phase was then discarded. The remaining organic phase was evaporated to dryness (Savant Speed Vac concentrator-evaporator 100H, Emerston Instruments, Scarborough, Canada). The residue was dissolved in 100 uL 50 mM triethylamine in acetonitrile. To this mixture was added, at 30 s intervals, 50 uL 60 mM ethyl chloroformate in acetonitrile, and 50 uL of a mixture of 1 M L-leucinamide hydrochloride (Sigma, St. Louis, Mo., USA) and 1 M triethylamine in methanol. After 2 min., 50 uL of water was added. Aliquots of 10 to 40 uL of the solution were injected into an HPLC.

Conjugated KT was analyzed in 100 to 500 uL urine after alkaline hydrolysis with 25 to 125 uL, respectively, of 1 M sodium hydroxide. The samples were then acidified with 0.6 M sulfuric acid using 100 uL in excess of the 1 M sodium hydroxide volume used for hydrolysis. The sample preparation was then carried out as described for plasma.

2.2.2. Other drugs tested

Fenoprofen (Eli Lilly, Toronto, Canada), flurbiprofen (Boots, U.K.), ibuprofen (Upjohn, Don Mills, Canada), naproxen (Syntex, Palo Alto, CA, U.S.A.), etodolac (Ayerst, Montreal, Canada), and tiaprofenic acid (Roussel, Montreal, Canada) were also subjected to

derivatization with L-leucinamide using the above sample preparation method.

2.2.3. Dosing

A 19 year-old (77.8 kg) healthy subject received 50 mg KT (Orudis, Rhone-Poulenc, Montreal, Canada) and donated 4 ml venous blood. The blood was collected 2 and 12 h after the dose into a heparinized Vacutainer, centrifuged, the plasma portion separated and stored at -20° until analysed. Urine collected from 12 to 24 h was also analyzed.

The study was approved by the Medical Ethics Committee of the University of Alberta Hospital and a written consent form was signed by the subject.

2.2.4. Standard curves

The enantiomers were quantified against sets of standard solutions prepared by spiking aliquots of 0.5 mL blank plasma with racemic KT (Rhone Poulenc, Montreal, Canada). Final concentrations of the enantiomers were 0.05, 0.1, 0.25, 0.5, 1.0, 2.5 and 5.0 mg/L. Aliquots of 0.5 mL urine were similarly spiked with KT to give final concentrations of 0.5, 2.5 and 5.0 mg/L. The solutions were subjected to analysis according to the method described for samples.

2.2.5 Extraction efficiency

To assess the efficiency of the extraction method, spiked solutions of racemic KT (1.0 and 5.0 mg/L in plasma and 1.0 and 10.0 mg/L in urine) were extracted (n=3) in the absence of IS using the

above method. After evaporation of the chloroformic layer, the extracted racemic KT was dissolved in 200 uL methanol and 100 uL IS solution was added. Aliquots of 10 - 40 uL of these solutions were chromatographed according to a non-stereospecific HPLC assay.[8] The area ratios (racemic KT/IS) of the eluting peaks were compared with those of solutions of similar concentrations which were injected directly into the HPLC without extraction. The order of elution of the enantiomers was determined by testing the retention time of a peak eluted from a pure S(+)-KT (Rhone-Poulenc, Montreal, Canada) solution.

2.2.6. Instrumentation

The HPLC consisted of a Waters (Mississauga, Ont. Canada) Model 590 pump, Model 712 Wisp, Model 481 UV spectrophotometer, and a Model 3390A Hewlett-Packard (Avondale, PA, USA) integrator. At ambient temperature, a 10 cm reversed-phase column (Whatman Partisil 5 ODS-3, Whatman, Clifton, NJ, USA) attached to a 5 cm guard column packed with 37-53 um $C_{18}$ material was utilized throughout the experiment. The mobile phase, 0.06 M potassium dihydrogen phosphate; acetonitrile; triethylamine (64:36:.02) was pumped at a flow rate of 1.0 mL/min. and the detector wavelength was 275 nm. The peak area method (enantiomer/IS) was used to calculate response.

## 2.3. RESULTS AND DISCUSSION

Figure 2-1 depicts chromatograms of blank plasma, blank urine and a plasma sample spiked with 0.25 mg/L of each enantiomer. Diastereoisomers of both the R(-)- and S(+)-KT, and IS eluted at 9.8,

Fig. 2-1. HPLC of blank plasma (A), blank urine (B), and plasma spiked with 0.25 mg/L of each enantiomer of KT (C). Injection volumes: 40 uL for plasma and 20 uL for urine solutions. Attenuation: plasma, changed from 1 to 2 at 14.5 min; urine, 2. Peaks: 1, R(-)-KT; 2, S(+)-KT; 3, R(-)-IS; 4, S(+)-IS.

11.3, and 17.7, 19.9 min, respectively.

No interfering peaks were observed. An excellent linear correlation between peak area ratios (enantiomers/IS) and enantiomer concentrations was found ($r^2$ > 0.995) over the enantiomer concentration range of 0.05 to 5.0 mg/L for plasma and 0.5 to 5.0 mg/L for urine. The best-fit lines passing through the data points were described using the peak area ratio (Y), and the concentration in ug/mL (X), where Y = .4161X + .0067, and Y = .4243X - .0037 for R(-)- and S(+)-enantiomers in plasma, respectively. The standard curves describing urine samples were Y = .5813X + .0059, and Y = .5880X + .0422 for R(-)- and S(+)-enantiomers, respectively. The coefficient of variation (C.V.) over the examined concentration ranges for plasma and urine was less than 10%.

The 2 h plasma sample had 2.27 mg/L of R(-)- and 2.03 mg/L of S(+)-enantiomer, while the 12 h sample had 0.13 mg/L of each enantiomer (Fig. 2-2). A 12-24 h collection of urine contained 0.12 and 0.18 mg of the R(-)- and S(+)-enantiomer, respectively (Fig. 2-2). Based on these observations, the minimum quantifiable concentration (MQC) in plasma of 0.05 mg/L found for this assay was essential if the drug concentrations were to be followed for up to 12 h post-dosing. Bjorkman [7] set his MQC at 0.25 mg/L while he also reported KT enantiomer concentrations of approximately 0.1 mg/L 7 h after a single dose of KT. Our assay, therefore, offers a higher sensitivity over that reported by Bjorkman [7].

The extraction yield from plasma (peak-area ratio comparisons of extracted versus directly chromatographed solutions) for the racemic

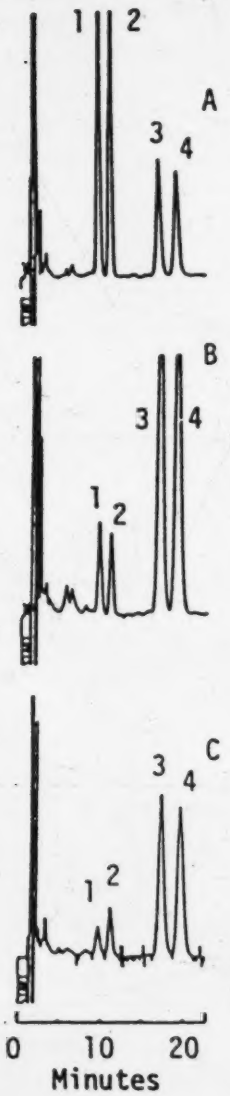


Fig. 2-2. HPLC of 2 h (A) and of 12 h (B) plasma samples, and of 12-24 h urine samples (C) after a 50 mg oral dose of KT. Injection volumes were 10, 40 and 40 uL for A,B and C, respectively. Attenuation: plasma, changed from 1 to 2 at 14.5 min; urine, 2. Peaks: 1, R(-)-KT; 2, S(+)-KT; 3, R(-)-IS; 4, S(+)-IS.

mixture was 77.58% and 78.15% for 1.0 and 5.0 mg/L, respectively. For urine samples, the yields were 75.51% and 73.96% for 1.0 and 10.0 mg/L racemic KT, respectively.

The maximum UV absorbance for derivatized KT enantiomers was 250 nm. However, as three small interfering peaks were consistently present at this wavelength, the assay was carried out at 275 nm. These interfering peaks which were also observed by Bjorkman during the analysis of indoprofen [6], and ketoprofen [7] seem to be a major problem in using the method originally utilized by Bjorkman [6]. For analysis of KT enantiomers, Bjorkman [7] used ion-pair extraction to avoid these peaks. By changing both the wavelength and method of extraction we also developed a simple and convenient HPLC method free of unwanted peaks. Additionally, preparation was rapid, as 12 samples required approximately 60 minutes.

It is worth mentioning that the applicability of this method was also tested for fenqprofen, flurbiprofen, ibuprofen, naproxen, etodolac and tiaprofenic acid. While all of the compounds formed diastereoisomers with L-leucinamide, satisfactory resolution of the enantiomers was noted only for fenoprofen and flurbiprofen under the conditions employed. With the exception of naproxen, peaks representing the enantiomers of other compounds did not interfere with those of R- and S-KT. This assay is stereospecific, convenient and suitable for determination of plasma and urine concentrations of KT following therapeutic doses.

## 2.4. REFERENCES


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# 3. Pharmacokinetics of Ketoprofen Enantiomers in Healthy Subjects Following Single and Multiple Doses*

## 3.1. INTRODUCTION

The stereoselective disposition of chiral compounds has recently been given much consideration.[1-9] Interest in enantioselective pharmacokinetics has been generated from the realization that constituent enantiomers of a chiral compound may exhibit different pharmacologic and toxicologic properties.[5-8] An important group of chiral compounds, the 2-arylpropionic acid type (2-APA) non-steroidal antiinflammatory drugs (NSAIDs), are commonly used in the treatment of joint and connective tissue disorders. Many of the 2-APAs exhibit stereoselective disposition kinetics and also undergo a unidirectional bioinversion of the less active R- to the active S-enantiomer.[5-8] It therefore follows that, although there is a wealth of information regarding the total drug concentration (R- plus S-enantiomer), this knowledge cannot be extrapolated to individual enantiomers, and may in fact, be misleading.[7,8]

Several studies detailing ketoprofen (KT) disposition kinetics in humans have been reported.[10, 11] However, none of these studies

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used stereospecific techniques. In this paper, we report the pharmacokinetics of ketoprofen enantiomers, following single and multiple doses, using a sensitive, convenient and enantiospecific HPLC method.[12]

## 3.2. METHODS

### 3.2.1. Drug administration and sample collection

The project was conducted in accordance with the principles of the Declaration of Helsinki, and was performed following approval by the Institutional Review Committee.

Eight healthy volunteers (Table 3-1), participated in the study after giving written informed consent. On the first morning of the study (at 0800 h) following an overnight fast, subjects were given 50 mg racemic KT (Rhone-Poulenc Pharm. Inc., Montreal, Canada) with a glass of water. No food was allowed for two h after dosing. Venous blood (4-5 mL) was collected into heparinized tubes at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8 and 12 h from an indwelling catheter inserted in a forearm vein. Urine (total output) was collected at 0, 3, 6, 12 and 24 h. The day after single dose administration, KT was given as 50 mg q6h for a total of 13 doses. After administration of the final dose, plasma and urine samples were again collected in the manner described above. Blood samples were centrifuged and plasma separated. The specimens were stored at -20° until analyzed, using a previously reported enantiospecific HPLC assay.[12]

No other drugs were allowed seven days prior to, or during the study period.

3.2.2. Treatment of data

Plasma enantiomer concentrations were plotted *versus* time. The area under the plasma KT concentration curve (AUC) from time of administration to 12 h (after single dose), and from time of administration to 6 h (following multiple dosing), was calculated using the linear trapezoidal method. For the single dose AUC, the area from the last data point to infinity ($C_{last}/\beta$) was added to $AUC_{0-12}$. The apparent elimination rate constant, $\beta$, was calculated from the best-fit line through the log-linear terminal phase of the curves. The plasma clearance (Cl/F=dose/AUC), and volume of distribution ($Vd/F=Cl/\beta$) were calculated for R-KT considering the dose to be equal to one-half of the administered racemate, and F to be the fraction of the dose absorbed. As the possibility of R- to S-isomer inversion could not be ruled out, and consequently the fraction of the dose entering the systemic circulation as S-KT remained unknown, Cl and Vd of the S-enantiomer could not be calculated. Half-life (t½) was calculated as $0.693/\beta$. The extent of conjugation in plasma and in urine was calculated by subtracting unchanged from the total (unchanged plus conjugated) KT before and after hydrolysis, respectively. Differences between the pharmacokinetic parameters of the individual enantiomers were assessed using the Student's paired *t-test*, at $\alpha=0.05$. The data are expressed as mean±SD.

Table 3-1. Subject Characteristics

| Subject | Age, years | Sex | Weight, kg | Creatinine Clearance mL/min |
|---|---|---|---|---|
| 1 | 27 | M | 85.0 | 131 |
| 2 | 27 | F | 54.7 | 84 |
| 3 | 25 | F | 76.1 | 88 |
| 4 | 22 | M | 57.8 | 112 |
| 5 | 19 | M | 77.8 | 142 |
| 6 | 18 | M | 72.7 | 123 |
| 7 | 26 | F | 67.1 | 103 |
| 8 | 26 | M | 67.4 | 86 |
| Mean | 24 | | 69.8 | 109 |
| SD | 3.6 | | 10.2 | 22 |

### 3.3. RESULTS

Figure 3-1 depicts the plasma KT enantiomers concentrations *versus* time curve following single and multiple doses of 50 mg racemic KT in subject 4 as representative of the sample population. Tables 3-2 and 3-3 contain pharmacokinetic parameters of S- and R-KT after single and multiple doses, respectively. In plasma, the concentration of R-KT tends to be consistently greater than S-KT. The observed difference, although small, was significant in all samples with the exception of those taken at 0.5 and 6 h following single doses, and 0.25 and 0.5 h following multiple doses. The peak plasma concentrations ($C_{max}$) were also significantly greater for R-KT as compared to S-KT following multiple doses. However, after the single dosing regimen, this difference in $C_{max}$ values was only significant if subject 5 was deleted from the group.

When examining the AUC of the enantiomers, a small but significant difference was observed, owing to the fact that the AUC of R-KT was consistently greater than that of S-KT. However, t½s of the enantiomers were not significantly different.

The mean of the ratios of S:R-KT concentrations throughout the entire collection times for plasma was $0.81\pm0.19$ and $0.87\pm0.11$, following single and multiple dosing, respectively. The mean S:R AUC ratios following single and multiple dosing were $0.85\pm0.09$, and $0.91\pm0.04$, respectively.

There were no significant differences between the pharmacokinetic parameters when comparing single and multiple dosing regimens. Peak plasma concentrations were rapidly achieved ($T_{max}$) in all subjects

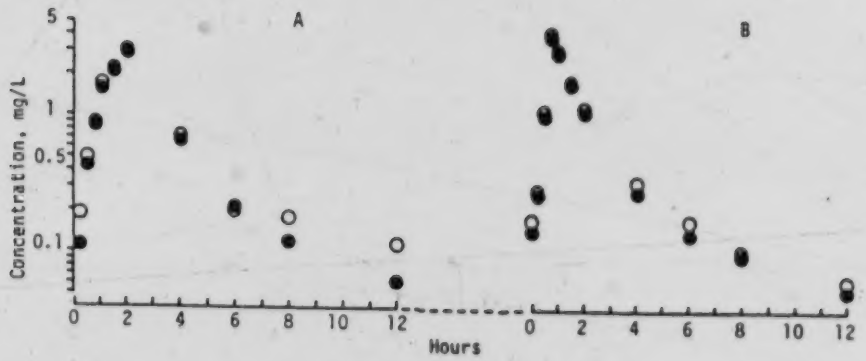


Fig. 3-1. Plasma S-( ● ) and R-( ○ )ketoprofen concentration *versus* time curves following single (A) and multiple (B) 50 mg doses in subject 4.

Table 3-2. Pharmacokinetic Parameters Following a Single 50 mg Dose of Racemic Ketoprofen

| Subject | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Mean (SD) |
|---|---|---|---|---|---|---|---|---|---|
| Tmax, h | | | | | | | | | |
| S | 2.0 | 0.75 | 0.75 | 2.0 | 2.0 | 0.5 | 1.0 | 1.5 | 1.3 (0.64) |
| R | 2.0 | 0.75 | 0.75 | 2.0 | 2.0 | 0.5 | 1.0 | 1.5 | 1.3 (0.64) |
| Cmax, mg/L | | | | | | | | | |
| S | 1.78 | 4.10 | 3.62 | 2.92 | 2.03 | 3.90 | 2.94 | 3.85 | 3.14 (0.88) |
| R | 1.91 | 4.13 | 3.69 | 3.11 | 2.27 | 3.94 | 3.24 | 4.02 | 3.29 (0.83) |
| t½, h | | | | | | | | | |
| S | 2.19 | 2.00 | 2.26 | 3.29 | 3.27 | 3.28 | 2.35 | 1.44 | 2.51 (0.69) |
| R | 2.90 | 2.46 | 3.01 | 3.56 | 4.71 | 8.25 | 2.21 | 3.29 | 3.80 (1.95) |
| AUC, (mg/L)h | | | | | | | | | |
| S | 4.52 | 5.73 | 6.11 | 8.19 | 5.73 | 6.43 | 4.35 | 6.31 | 5.92 (1.20) |
| R | 5.40 | 6.39 | 6.17 | 9.11 | 6.91 | 9.41 | 5.10 | 7.53 | 7.00 (1.59)[a] |
| Vd/F, L | | | | | | | | | |
| R | 19.3 | 14.0 | 17.6 | 14.4 | 24.1 | 31.7 | 15.8 | 15.8 | 19.1 (6.04) |
| Cl/F, L/h | | | | | | | | | |
| R | 4.63 | 3.91 | 4.05 | 2.74 | 3.62 | 2.66 | 4.90 | 3.32 | 3.73 (0.81) |
| ΣXu0-24, mg | | | | | | | | | |
| S | 16.9 | 24.7 | 20.6 | 12.5 | 21.0 | 36.1 | 20.9 | 22.2 | 21.9 (6.82) |
| R | 13.3 | 21.2 | 26.8 | 10.3 | 17.9 | 30.4 | 18.3 | 19.9 | 18.5 (5.95)[a] |

[a] Significantly different from the S-enantiomer.

Table 3-3. Pharmacokinetic Parameters Following Multiple Doses (50 mg q6h)

| Subject | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | Mean (SD) |
|---|---|---|---|---|---|---|---|---|---|
| Tmax, h | | | | | | | | | |
| S | 4.0 | 0.75 | 0.75 | 0.75 | 2.0 | 0.5 | 0.75 | 1.5 | 1.4 (1.2) |
| R | 4.0 | 0.75 | 0.75 | 0.75 | 2.0 | 0.5 | 0.75 | 1.5 | 1.4 (1.2) |
| Cmax, mg/L | | | | | | | | | |
| S | 0.64 | 2.53 | 3.22 | 4.01 | 1.97 | 2.77 | 2.88 | 3.18 | 2.65 (1.00) |
| R | 0.73 | 2.57 | 3.29 | 4.08 | 2.35 | 2.78 | 3.18 | 3.39 | 2.80 (0.99)[a] |
| t½, h | | | | | | | | | |
| S | 2.66 | 3.15 | 2.53 | 4.66 | 2.07 | 3.39 | 4.00 | 3.11 | 3.19 (0.82) |
| R | 3.32 | 3.79 | 3.46 | 4.28 | 2.46 | 5.50 | 3.48 | 3.96 | 3.78 (0.88) |
| AUC, (mg/L)h | | | | | | | | | |
| S | 2.60 | 4.57 | 5.22 | 5.69 | 10.7 | 4.46 | 3.47 | 6.58 | 5.41 (2.46) |
| R | 2.97 | 5.04 | 5.30 | 6.11 | 1199 | 4.71 | 4.09 | 7.33 | 5.93 (2.74)[a] |
| Vd/F, L | | | | | | | | | |
| R | 40.1 | 27.6 | 23.5 | 25.6 | 7.47 | 42.1 | 40.7 | 18.9 | 21.5 (11.6) |
| Cl/F, L/h | | | | | | | | | |
| R | 8.42 | 4.96 | 4.72 | 4.09 | 2.10 | 5.31 | 6.11 | 3.41 | 4.89 (1.88) |
| ΣXuO-6, mg | | | | | | | | | |
| S | 19.1 | 23.3 | 19.5 | 20.8 | 21.3 | 17.6 | 17.8 | 29.1 | 21.2 (4.74) |
| R | 15.6 | 19.0 | 16.2 | 18.4 | 18.9 | 15.1 | 15.2 | 26.4 | 18.1 (3.74)[a] |

[a] Significantly different from the S-enantiomer.

(Tables 3-2 and 3-3), except one, where a 4 hour lag-time was observed after multiple doses.

Only negligible amounts of intact enantiomers were found in urine. The cumulative amount of conjugated S-KT excreted in urine was 21.9±6.82, and 21.1±4.74 mg following single and multiple dosing, respectively. The cumulative amount of conjugated R-KT was significantly less being 18.5±5.95, and 18.1±3.74 mg following single and multiple dosing, respectively. The mean S:R ratios of cumulative conjugated KT following single and multiple dosing were 1.19±0.05 and 1.17±0.05, respectively.

### 3.3.4. DISCUSSION

The observed mean t½ of S- and R-enantiomers were 2.51±0.69 and 3.80±1.95 h (single dose), and 3.19±0.82 and 3.78±0.88 h (multiple doses), respectively. These values are longer than 1.13±0.07[10] and 1.5 h[13] reported by others for a racemic mixture of the enantiomers. The reason for this discrepancy may be due to the fact that owing to the sensitivity of our assay, we were able to follow plasma KT concentrations for as long as 12 h as opposed to approximately 8 h followed by others. A slower decline in plasma concentration of both enantiomers followed the initial rapid disappearance suggesting multi-exponential disposition kinetics. It is important to note that the true terminal t½ of KT enantiomers may be even longer than these reported here as, due to the limited data points, the terminal slope of the concentration-time curve may have been overestimated. In fact, Delbarre *et al.*,[14] using tritiated KT, showed an initial t½ of 1.4 h, followed by a terminal t½ of 1.2 days.

It is documented that for many of the 2-APAs, there is a significant difference in the disposition of the two enantiomers. The usual case is that the S- predominates over the less active R-enantiomer in plasma.[6] Recently, it has been shown that tiaprofenic acid does not exhibit stereoselective pharmacokinetics. In fact, the plasma, synovial fluid and urinary levels of the enantiomers of this drug were superimposable.[3] Ketoprofen appears to be another exception to this generalization. Although we observed a very small, but significant difference in the disposition of the enantiomers, the R-and not the S-enantiomer was the predominant isomer in plasma. However, the S-enantiomer was more prevalent in urine. While the S:R-KT concentration ratios in plasma were, on average, 19 and 13% less than unity, in urine the ratios were 19 and 17% more than unity following single and multiple dosing, respectively.

The observed differences in the disposition kinetics of KT enantiomers may be attributed to various mechanisms. The major elimination pathway of KT is through ester glucuronidation. In healthy subjects, the formed conjugates are readily excreted through the renal and/or biliary routes with very little systemic accumulation.[15] We observed conjugated:unchanged KT plasma concentration ratios to be 0.12 and 0.07 for S- and R-KT, respectively. The greater plasma intact R-KT concentration coupled with the observed less extensive urinary excretion of the conjugated R-KT as compared to S-KT seem to indicate preferential conjugation in favor of the S-enantiomer. However, considering that over 80% of the drug is eliminated through conjugation, an enantiomer with greater

affinity for conjugation is expected to be eliminated with a t½ significantly shorter than the other. The proposed stereoselective conjugation, however, may not have been extensive enough to result in significant differences in the enantiomers t½ (Tables 3-2 and 3-3).

The urinary recovery of the given doses averaged 82% (44% as S- and 38% as R-conjugated KT). The unrecovered 18% may have found its way into the bile. If so, it becomes plausible to suggest preferential biliary excretion as another reason for the observed stereoselectivity in urinary excretion of KT enantiomers. As compared to S-KT, R-KT may be excreted more in bile and less in urine. The biliary excretion of acidic compounds may be an active, and hence competitive process.[16] Therefore, conjugated KT enantiomers may compete for biliary excretion. The extent of such a competition may become greater and more clinically relevant if the renal route became partly blocked and consequently the biliary pathway emerges as the major route.[17] This hypothesis is currently being tested in elderly arthritic patients in whom the kidney function is reduced.

The metabolic inversion of one enantiomer to another[5] may also be another possible mechanism giving rise to stereoselective pharmacokinetics. However, proof of inversion can only be unequivocally acceptable if pure enantiomers are administered, or if more than 50% of a racemic dose is recovered as one of the enantiomers, i.e., recovery > dose. The possibility of an inversion, therefore, cannot be ruled out based on data presented here. The extent of inversion (if any), however, cannot be substantial as only small differences between the two enantiomers were observed in urine.

In subjects with normal kidney function, the significant differences in the pharmacokinetics of KT enantiomers are so small that clinically they may be considered negligible. However, it has been reported that kidney dysfunction significantly reduces the clearance of conjugated drugs such as KT[18], and it has been suggested that in such conditions, the biliary pathway may become more involved in elimination of the drug.[17] Hence, preferential renal or biliary eliminations of these labile conjugates may result in preferential accumulation and then hydrolysis of the active or less active enantiomers in the body of these patients. These hypotheses are being tested in our laboratory.

## 3.5. REFERENCES


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# 4. PHARMACOKINETICS OF KETOPROFEN ENANTIOMERS IN YOUNG AND ELDERLY ARTHRITIC PATIENTS FOLLOWING SINGLE AND MULTIPLE DOSES*

## 4.1. INTRODUCTION

Recently, much attention has been directed toward stereoselective pharmacokinetics of chiral compounds.[1-11] This interest, in part, has included investigation of the 2-arylpropionic acid (2-APA) non-steroidal antiinflammatory drugs (NSAIDs). Many of the 2-APA NSAIDs not only demonstrate enantioselectivity in their kinetic disposition, but also exhibit a unidirectional bioinversion of the less active R- to the more active S-enantiomer. It therefore follows that investigation of these compounds must involve a stereospecific assay, as data generated from methods measuring total (R- plus S-enantiomer) concentrations may be misleading.

We have recently developed a stereospecific HPLC method for the assay of ketoprofen (KT) enantiomers in plasma and urine.[12] This method was applied to determine the pharmacokinetics of KT enantiomers in healthy subjects following single and multiple doses.[11] As the disposition kinetics of KT have been shown to be

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different in the elderly,[13] we decided to include elderly, as well as young patients in our study. Moreover, as the effects of arthritis on the pharmacokinetics of KT are not established, we examined arthritic patients. In this paper, we report the pharmacokinetics of KT enantiomers in young and in elderly arthritic patients following single and multiple doses, using the previously reported HPLC method.[12]

## 4.2. METHODS

### 4.2.1. Drug administration and sample collection

The project was conducted in accordance with the principles of the Declaration of Helsinki. Approval of the Institutional Review Committee was obtained prior to conducting the study.

A total of nine young (44±12 years), and nine elderly (72±4.6 years) arthritic patients (Table 4-1) were recruited by the Division of Rheumatology, University of Alberta. Patients with rheumatoid arthritis (RA) or osteoarthritis (OA) were included in the study, after giving written informed consent.

Following an overnight fast, each patient received 50 mg racemic KT capsules (Rhone-Poulenc Pharma. Inc., Montreal, Canada) at 0800 h, along with one glass of water (200 mL). No food was allowed for two h after dosing. After administration of KT, venous blood (4 - 5 mL) was collected via an indwelling catheter inserted in a forearm vein into heparinized tubes at 0, 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0, and 12 h. Urine (total output) was collected on the same

day, at intervals of 0-3, 3-6, 6-12, and 12-24 h. On another occassion, KT was administered in multiple doses given as 50 mg q6h for a total of 13 doses. Plasma samples were collected in the manner previously described, following administration of the final dose. Urine (total output) was again collected from 0-3, and from 3-6 h. After collection of blood samples, plasma was separated by centrifugation. All specimens were stored at -20° prior to analysis. No other NSAIDs were allowed during, or two days prior to the study.

4.2.2. Determination of unchanged and conjugated drug

Plasma and urinary concentrations of KT enantiomers present as intact and ester conjugates were measured using a stereospecific assay.[12] Samples were assayed before and after alkaline hydrolysis (1 M NaOH) and the difference between the two readings was counted as the concentration of the conjugated KT enantiomers.

4.2.3. Treatment of Data

KT enantiomer concentrations were plotted *versus* time. Following single dose administration, the area under the plasma drug concentration curve was calculated from time of administration to infinity by the linear trapezoidal rule, where the area from the last data point ($C_{last}$) to infinity was calculated by $C_{last}/\beta$. The apparent elimination rate constant, $\beta$, was calculated from the best-fit line passing through the last three data points of the log-linear terminal phase of the curve. Half-life ($t_{½}$) was calculated as $0.693/\beta$. Plasma clearance (Cl/F=DOSE/AUC) and volume

Table 4-1. Patient Characteristics

| Patient | Age (years) | Sex | Weight (kg) | Creatinine Clearance (mL/min) | Diagnosis |
|---|---|---|---|---|---|
| YOUNG | | | | | |
| 1 | 43 | F | 74.2 | 125 | OA |
| 2 | 52 | F | 57.9 | 113 | RA |
| 3 | 54 | M | 62.1 | 99 | RA |
| 4 | 55 | F | 79.9 | 84 | OA |
| 5 | 47 | F | 64.7 | 73 | RA |
| 6 | 34 | F | 71.5 | 106 | OA |
| 7 | 55 | F | 56.1 | 66 | RA |
| 8 | 35 | F | 58.2 | 81 | RA |
| 9 | 20 | F | 70.6 | 113 | OA |
| Mean | 44 | | 66.1 | 96 | |
| SD | 12 | | 8.3 | 20 | |
| ELDERLY | | | | | |
| 10 | 71 | F | 41.4 | 43 | RA |
| 11 | 78 | F | 70.5 | 52 | RA |
| 12 | 73 | M | 66.2 | 47 | RA |
| 13 | 74 | F | 48.5 | 34 | OA |
| 14 | 71 | F | 58.3 | 47 | RA |
| 15 | 66 | F | 63.1 | 65 | RA |
| 16 | 78 | F | 81.2 | 56 | OA |
| 17 | 74 | M | 60.8 | 53 | RA |
| 18 | 65 | F | 66.9 | 61 | OA |
| Mean | 72 | | 61.9 | 51 | |
| SD | 4.6 | | 11.8 | 9.4 | |

of distribution ($Vd/F=Cl/\beta$) were calculated for R-KT, where the dose was considered to be one-half of the administered racemate. The fraction of the dose absorbed, F, was included in both Cl and Vd terms. Plasma clearance and volume of distributuion were not calculated for S-KT, as the possibility of R- to S-bioinversion could not be ruled out. Consequently, the exact amount of S-KT entering the systemic circulation was unknown. Renal clearance ($Cl_r$) was calculated where $Cl_r=\Sigma Xu/AUC$. For this calculation, $\Sigma Xu$ was the cumulative amount of conjugated KT excreted in urine and AUC was the corresponding value for conjugated KT in plasma.

The above calculations also applied following administration of multiple doses. However, the one exception was calculation of AUC. In this instance, AUC was calculated from time of KT administration to 6 hours.

The cumulative amount of drug excreted in urine was calculated from 0-24 h following single dosing, as elimination of KT was complete within 24 hours. Following multiple doses, the urinary cumulative amount was calculated from 0-6 hours.

The paired Student's t-test ($\alpha=0.05$) was used to examine the differences in pharmacokinetic parameters between R- and S-enantiomers within groups. Differences between groups were examined using a one-way analysis of variance. The data are expressed as mean±SD.

## 4.3. RESULTS

### 4.3.1. Young patients

Urine data from patient 3 could not be used, as peaks interfering

with KT were noticed.

Plasma concentrations of KT enantiomers *versus* time following single and multiple doses in patient 7 are depicted in figure 4-1, as this patient closely represents the mean of all the young patients. Pharmacokinetic parameters pertaining to young arthritic patients are summarized in Tables 4-2 and 4-3. Although the $C_{max}$ for S-KT was usually less than that of R-KT, this difference only showed significance after single dose administration. Similarly, the mean AUC and $t_{½}$ of R-KT seemed to be greater than that of S-KT following both single and multiple doses. The differences, however, were not significant.

There were no significant differences in any of the pharmacokinetic parameters when single *versus* multiple doses were compared. Accumulation of drug following multiple dose administration was negligible.

After single dose administration, conjugated KT enantiomers in plasma at $T_{max}$ constituted from 0 to 14%, and from 0 to 5% of the total (intact plus conjugated) S- and R-KT, respectively. Similarly, following multiple doses, these values were 0 to 14%, and 0 to 4% of total S- and R-KT, respectively. Samples collected at other than $T_{max}$ generally contained negligible concentrations of conjugated enantiomers.

The KT enantiomers excreted in urine are present almost exclusively as readily hydrolyzable conjugates. Differences in the cumulative amount of conjugated S- and R-KT excreted in urine are statistically significant. The mean S:R ratios of cumulative conjugated KT enantiomers in urine were 1.30($\pm$0.11)and 1.29($\pm$0.07)

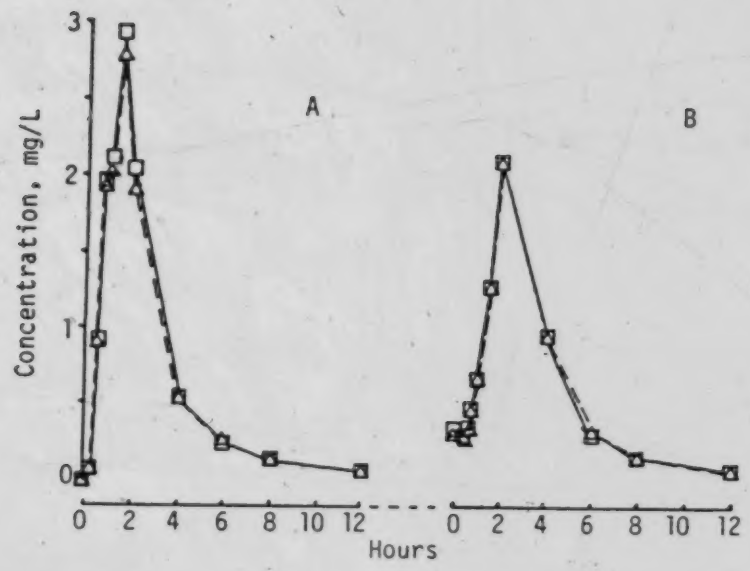


Fig. 4-1. Plasma S-(△) and R-(□) intact ketoprofen concentration *versus* time curves following single (A) and multiple (B) 50 mg doses in subject 7 (young arthritic). The plasma concentrations of the conjugated enantiomers were negligible.

TABLE 4-2. Pharmacokinetic Parameters Following a Single 50 mg Dose of Racemic Ketoprofen to Young Patients

| Patient | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | Mean (SD) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $T_{max}$, h | | | | | | | | | | | |
| | S | 0.75 | 0.75 | 0.5 | 1.5 | 1.0 | 1.5 | 1.5 | 1.5 | 0.5 | 1.1 (0.5) |
| | R | 0.75 | 0.75 | 0.5 | 1.5 | 1.0 | 1.5 | 1.5 | 1.5 | 0.5 | 1.1 (0.5) |
| $C_{max}$, mg/L | | | | | | | | | | | |
| | S | 2.74 | 3.14 | 2.31 | 3.12 | 0.83 | 0.83 | 2.79 | 1.99 | 3.08 | 2.31 (0.92) |
| | R | 2.79 | 3.36 | 2.29 | 3.32 | 0.88 | 0.88 | 2.93 | 1.96 | 3.10 | 2.39 (0.97)[a] |
| AUC, (mg/L)h | | | | | | | | | | | |
| | S | 5.52 | 3.32 | 6.69 | 7.58 | 4.56 | 4.08 | 7.56 | 4.62 | 5.51 | 5.49 (1.52) |
| | R | 5.52 | 4.13 | 5.73 | 9.08 | 4.52 | 4.27 | 7.52 | 4.21 | 5.77 | 5.64 (1.69) |
| t½, h | | | | | | | | | | | |
| | S | 2.76 | 2.57 | 3.61 | 2.89 | 2.57 | 2.68 | 2.50 | 1.55 | 2.52 | 2.63 (0.53) |
| | R | 2.44 | 4.98 | 3.50 | 4.97 | 2.00 | 3.07 | 3.45 | 1.84 | 2.44 | 3.19 (1.17) |
| Vd/F, L | | | | | | | | | | | |
| | R | 16.18 | 43.21 | 20.60 | 19.64 | 15.80 | 25.43 | 16.60 | 15.63 | 15.46 | 20.95 (8.97) |
| Cl/F, L/h | | | | | | | | | | | |
| | R | 4.53 | 6.05 | 4.12 | 2.75 | 5.53 | 5.85 | 3.32 | 5.94 | 4.33 | 4.71 (1.20) |
| ΣXu, mg | | | | | | | | | | | |
| | S | 24.39 | 20.89 | - | 15.72 | 11.87 | 41.94 | 13.88 | 19.52 | 26.44 | 21.83 (9.55) |
| | R | 20.77 | 17.35 | - | 12.07 | 9.43 | 32.94 | 10.31 | 15.26 | 19.17 | 17.16 (7.58)[a] |

[a] statistically different from S enantiomer

Table 4-3. Pharmacokinetic Following Multiple Doses (50 mg q6h) of Racemic Ketoprofen to Young Patients

| Patient | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | MEAN (SD) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $T_{max}$, h | | | | | | | | | | | |
| | S | 2.0 | 0.5 | 2.0 | 0.75 | 2.0 | 0.5 | 2.0 | 1.0 | 0.75 | 1.3 (0.7) |
| | R | 2.0 | 0.5 | 2.0 | 0.75 | 2.0 | 0.5 | 2.0 | 1.0 | 0.75 | 1.3 (0.7) |
| $C_{max}$, mg/L | | | | | | | | | | | |
| | S | 2.38 | 3.92 | 1.68 | 3.12 | 2.65 | 3.46 | 2.11 | 3.67 | 3.14 | 2.90 (0.75) |
| | R | 2.57 | 3.96 | 1.68 | 3.18 | 2.78 | 3.46 | 2.10 | 3.33 | 3.20 | 2.92 (0.71) |
| AUC, (mg/L)h | | | | | | | | | | | |
| | S | 5.04 | 3.55 | 4.22 | 6.57 | 6.23 | 5.46 | 5.96 | 5.57 | 5.87 | 5.39 (0.97) |
| | R | 5.18 | 3.92 | 3.98 | 6.62 | 6.47 | 5.47 | 6.01 | 4.26 | 6.21 | 5.35 (1.07) |
| t½, h | | | | | | | | | | | |
| | S | 1.95 | 3.14 | 3.41 | 3.18 | 3.36 | 3.72 | 2.65 | 2.55 | 4.82 | 3.20 (0.81) |
| | R | 3.06 | 2.53 | 3.17 | 5.06 | 2.50 | 3.52 | 3.18 | 1.93 | 2.73 | 3.08 (0.88) |
| Vd/F, L | | | | | | | | | | | |
| | R | 21.00 | 23.63 | 28.55 | 27.00 | 13.79 | 21.55 | 18.91 | 16.31 | 16.12 | 20.76 (5.02) |
| Cl/F, L/h | | | | | | | | | | | |
| | R | 4.83 | 6.38 | 6.28 | 3.78 | 3.86 | 4.31 | 4.16 | 5.87 | 4.03 | 4.83 (1.06) |
| ΣXu, mg | | | | | | | | | | | |
| | S | 27.99 | 5.84 | - | 16.92 | 17.30 | 42.75 | 28.15 | 26.50 | 24.02 | 23.68 (10.79) |
| | R | 22.98 | 4.42 | - | 12.70 | 13.48 | 32.51 | 21.24 | 19.27 | 20.59 | 18.40 (8.33)[a] |

[a]statistically different from S enantiomer

following single and multiple doses, respectively.

4.3.2. Elderly patients

Of the 9 elderly patients, one patient (# 10) had an interfering peak in plasma and urine, but only following the single dose KT.

Figure 4-2 depicts plasma concentrations of KT enantiomers *versus* time from patient 13, who closely represents the mean of the sample population. Tables 4-4 and 4-5 summarize the pharmacokinetic data obtained from the elderly sample. As with the young patient sample, differences between S- and R-KT plasma concentration following either single or multiple doses were insignificant. AUCs of R-KT tended to be greater than for S-KT, however the differences were insignificant. Similarly, the $t_{½}$ values of S- and R-KT following either single or multiple doses were not significantly different.

Tables 4-6 and 4-7 summarize pharmacokinetic parameters regarding conjugated KT obtained from the elderly subjects after single and multiple doses, respectively. The data clearly indicate that the $C_{max}$ and the AUC of conjugated S-KT is significantly greater than R-KT. Conjugated drug in plasma (at $T_{max}$) constituted up to 30% and 26% for S- and R-KT, respectively, after administration of a single dose. Following multiple doses, conjugates in plasma were up to 58% and 45%, for S- and R-KT, respectively. Conjugated KT enantiomers in plasma obtained from patient 13 are depicted in figure 4-2.

As observed with the young group, a significant difference between cumulative urinary excretion of conjugated S- and R-KT was

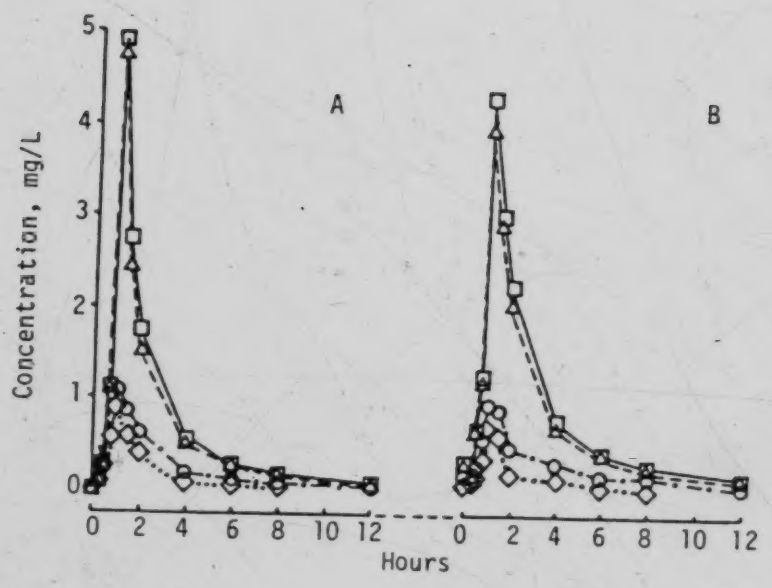


Fig. 4-2 Plasma time courses of the intact and conjugated ketoprofen in an elderly arthritic patient (# 13). Key: △, S; □, R; ○, conjugated S; ◇, conjugated R.

Table 4-4. Pharmacokinetic Parameters Following a Single 50 mg Dose of Racemic Ketoprofen in Elderly Patients

| Patient | | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | MEAN (SD) |
|---|---|---|---|---|---|---|---|---|---|---|
| $T_{max}$, h | | | | | | | | | | |
| | S | 0.5 | 0.75 | 1.0 | 1.0 | 1.0 | 1.0 | 4.0 | 0.5 | 1.2 (1.2) |
| | R | 0.5 | 0.75 | 1.0 | 1.0 | 1.0 | 1.0 | 4.0 | 0.5 | 1.2 (1.2) |
| $C_{max}$, mg/L | | | | | | | | | | |
| | S | 3.87 | 2.90 | 2.97 | 4.75 | 3.10 | 2.79 | 0.88 | 3.02 | 3.01 (1.09) |
| | R | 3.94 | 2.96 | 3.09 | 4.90 | 3.08 | 2.75 | 0.94 | 3.35 | 3.13 (1.12) |
| AUC, (mg/L)h | | | | | | | | | | |
| | S | 6.87 | 7.00 | 9.88 | 7.72 | 5.79 | 7.48 | 5.45 | 5.46 | 6.96 (1.48) |
| | R | 7.78 | 7.71 | 10.29 | 8.69 | 5.14 | 6.43 | 5.57 | 6.69 | 7.29 (1.69) |
| $t_{½}$, h | | | | | | | | | | |
| | S | 3.31 | 3.90 | 3.11 | 3.05 | 2.33 | 3.86 | 3.23 | 3.36 | 3.27 (0.49) |
| | R | 4.26 | 3.67 | 2.93 | 3.61 | 1.63 | 2.68 | 3.13 | 3.21 | 3.14 (0.78) |
| Vd/F, L | | | | | | | | | | |
| | R | 20.06 | 17.05 | 10.13 | 15.16 | 11.30 | 14.96 | 20.41 | 17.00 | 15.76 (3.70) |
| Cl/F, L/h | | | | | | | | | | |
| | R | 3.21 | 3.24 | 2.43 | 2.88 | 4.86 | 3.89 | 4.49 | 3.74 | 3.59 (0.82) |
| $\Sigma Xu$, mg | | | | | | | | | | |
| | S | 10.45 | 33.85 | 17.13 | 12.78 | 21.05 | 11.30 | 15.38 | 17.67 | 17.45 (7.51) |
| | R | 7.04 | 26.71 | 13.06 | 9.68 | 15.41 | 8.71 | 11.35 | 13.85 | 13.23 (6.12)[a] |

[a] statistically different from S enantiomer

Table 4-5. Pharmacokinetic Parameters Following Multiple Doses (50 mg q6h) of Racemic Ketoprofen in Elderly Patients

| Patient | | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | MEAN | (SD) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $T_{max}$, h | | | | | | | | | | | | |
| | S | 1.0 | 1.0 | 0.5 | 0 | 1.0 | 0.75 | 0.5 | 4.0 | 0.5 | 1.0 | (1.2) |
| | R | 1.0 | 1.0 | 0.5 | 0 | 1.0 | 0.75 | 0.5 | 4.0 | 0.75 | 1.1 | (1.2) |
| $C_{max}$, mg/L | | | | | | | | | | | | |
| | S | 3.10 | 3.57 | 3.40 | 3.52 | 4.24 | 3.57 | 4.34 | 0.59 | 2.98 | 3.26 | (1.10) |
| | R | 3.26 | 3.55 | 3.43 | 3.41 | 4.31 | 3.70 | 4.30 | 0.60 | 3.08 | 3.29 | (1.10) |
| AUC, (mg/L)h | | | | | | | | | | | | |
| | S | 7.45 | 5.72 | 7.46 | 5.72 | 7.92 | 5.29 | 7.81 | 2.76 | 5.42 | 6.17 | (1.67) |
| | R | 8.04 | 5.61 | 7.62 | 5.33 | 8.60 | 5.42 | 7.96 | 2.70 | 6.21 | 6.39 | (1.87) |
| t½, h | | | | | | | | | | | | |
| | S | 4.18 | 3.55 | 3.87 | 3.92 | 5.11 | 4.36 | 3.31 | 1.55 | 4.69 | 3.84 | (1.02) |
| | R | 4.59 | 3.86 | 3.63 | 4.92 | 4.32 | 3.74 | 3.50 | 1.76 | 3.65 | 3.77 | (0.90) |
| Vd/F, L | | | | | | | | | | | | |
| | R | 20.73 | 24.78 | 17.26 | 33.50 | 18.19 | 24.26 | 15.70 | 13.59 | 21.21 | 21.02 | (5.98) |
| Cl/F, L/h | | | | | | | | | | | | |
| | R | 3.11 | 4.46 | 3.28 | 4.69 | 2.91 | 4.61 | 3.14 | 5.30 | 4.03 | 3.95 | (0.86) |
| ΣXu, mg | | | | | | | | | | | | |
| | S | | 11.76 | 25.14 | 14.21 | 15.14 | 10.97 | 13.44 | 6.13 | 19.63 | 14.55 | (5.75) |
| | R | | 8.02 | 19.25 | 9.44 | 11.59 | 9.40 | 8.25 | 4.59 | 14.83 | 10.67 | (4.55)[a] |

[a]statistically different from S enantiomer

Table 4-6. Pharmacokinetic Parameters of Conjugated KT in the Elderly Following a Single 50 mg Dose of Racemic Ketoprofen

| Patient | | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | MEAN | (SD) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| $T_{max}$, h | S | 1.0 | 1.0 | 1.0 | 1.0 | 1.5 | b | 4.0 | 0.75 | 1.5 | (1.1) |
| | R | 0.5 | 1.0 | 1.0 | 1.0 | 6.0 | b | b | 0.75 | 1.7 | (2.1) |
| $C_{max}$, mg/L | S | 0.94 | 0.53 | 0.90 | 1.14 | 0.35 | b | 0.15 | 0.67 | 0.67 | (0.35) |
| | R | 0.30 | 0.33 | 0.80 | 0.94 | 0.13 | b | b | 0.31 | 0.47 | (0.32)[a] |
| AUC, (mg/L)h | S | 2.48 | 1.99 | 3.25 | 2.70 | 1.17 | b | 0.90 | 3.24 | 2.25 | (0.94) |
| | R | 0.58 | 0.76 | 2.04 | 1.53 | 0.82 | b | b | 0.75 | 1.08 | (0.58)[a] |
| $t_{½}$, h | S | 5.34 | 2.77 | 2.10 | 3.09 | 1.52 | b | 6.75 | 5.40 | 3.85 | (1.97) |
| | R | 2.87 | 1.52 | 2.37 | 2.71 | 0.55 | b | b | 1.20 | 1.87 | (0.92)[a] |
| $Cl_r$, L/h | S | 4.21 | 17.01 | 5.27 | 4.14 | 13.85 | c | 17.09 | 5.45 | 9.57 | (6.11) |
| | R | 12.1 | 35.1 | 5.51 | 6.33 | 18.8 | c | c | 12.3 | 15.0 | (11.0)[a] |

[a] statistically different from S enantiomer
[b] not detectable
[c] unable to clearly determine

Table 4-7. Pharmacokinetic Parameters of Conjugated KT in the Elderly Following Multiple Doses (50 mg q6h)

| Patient | | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | MEAN | (SD) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $T_{max}$, h | | | | | | | | | | | | |
| | S | 1.5 | 1.0 | 1.0 | 0.25 | 1.0 | 1.5 | 0.5 | 4.0 | 1.0 | 1.3 | (1.1) |
| | R | 0.75 | 1.0 | 1.0 | 0 | 1.0 | c | c | 4.0 | 1.0 | 1.3 | (1.3) |
| $C_{max}$, mg/L | | | | | | | | | | | | |
| | S | 0.78 | 0.75 | 0.60 | 0.69 | 0.99 | 0.41 | 0.61 | 0.35 | 0.66 | 0.65 | (0.19 |
| | R | 0.50 | 0.38 | 0.36 | 0.39 | 0.79 | c | c | 0.17 | 0.22 | 0.40 | (0.20 |
| AUC, (mg/L)h | | | | | | | | | | | | |
| | S | 2.80 | 1.66 | 1.35 | 1.67 | 2.46 | 1.29 | 2.27 | 1.63 | 1.88 | 1.89 | (0.51 |
| | R | 1.69 | 0.34 | 0.41 | 0.57 | 1.33 | c | c | 0.92 | 0.25 | 0.61 | (0.59 |
| t½, h | | | | | | | | | | | | |
| | S | 6.46 | 4.32 | 1.99 | 2.04 | 3.15 | 2.01 | 10.97 | 8.84 | 7.3 | 5.23 | (3.32 |
| | R | 1.79 | 0.44 | 3.10 | 0.99 | 2.86 | c | c | b | 0.18 | 1.56 | (1.23 |
| $Cl_r$, L/h | | | | | | | | | | | | |
| | S | b | 7.08 | 18.6 | 8.51 | 6.15 | 8.50 | 5.22 | 3.76 | 10.4 | 8.62 | (4.52 |
| | R | b | 23.6 | 47.0 | 16.6 | 8.71 | b | b | 18.4 | 59.3 | 29.0 | (19.8 |

[a]statistically different from S enantiomer
[b]unable to clearly determine
[c]not detectable

noted following both single and multiple doses. The cumulative S:R ratios of conjugated KT excreted in urine were 1.34±0.06 and 1.38±0.14 following single and multiple doses, respectively. After single dosing, the $Cl_r$ was 9.57±6.11 and 15.0±11.0 L/h for conjugated S- and R-KT, respectively. The values obtained after multiple doses were 8.62±4.52 and 28.9±19.8 L/h for both S- and R-KT conjugates, respectively. In both cases, the differences between the two enantiomers were significant, where $Cl_r$ was greater for conjugated R-enantiomer.

## 4.4. DISCUSSION

We recently studied the stereoselective pharmacokinetics of KT in a group of young, healthy volunteers.[11] In those healthy subjects there were no significant differences between pharmacokinetic indices calculated after single and multiple doses. In plasma, small but significant differences were found between concentrations of the enantiomers (S/R, 0.81±0.19 after single and 0.87±0.11 after repeated doses). More than 80% of the given doses were found in urine as conjugated S- and R-KT, the predominant enantiomer being S-KT (S/R, 1.19±0.05 after single and 1.17±0.05 after repeated doses). It was suggested that stereoselective conjugation followed by preferential biliary excretion of the conjugated R-KT enantiomer was responsible for those observations. In both groups of patients studied in this work (Tables 4-2 to 4-5 and Fig. 4-1,4-2) the general pattern of KT disposition kinetics was similar to that observed in healthy subjects. Although not significant, differences were noticed between young and elderly patients. These included a greater $C_{max}$,

elevated AUC, prolonged t½ and smaller Vd in the elderly patients, for both enantiomers. Advenier *et al.*[13], using a nonstereospecific assay to study the pharmacokinetics of KT in the elderly, also made similar observations. The effect of age, however, was significant and more pronounced in the patients studied by Advenier *et al.*[13] as compared to the group reported here. They attributed their observations to a slower metabolism of the drug in the elderly.

Dose dependent elimination of KT may be an explanation for the discrepancy between the observation of Advenier *et al.*[13] and ours as they administered a 150 mg dose, i.e., 3-fold greater dose than ours. Interestingly, while only negligible concentrations of conjugated R- and S-KT were present in plasma of young healthy[11] and arthritic subjects, concentrations in plasma of elderly patients were substantial (Tables 4-6,4-7 and Fig. 4-2). Conjugated S-KT, however, was more prevalent than was conjugated R-KT. This indicates a substantial presence of the conjugated KT enantiomers (especially of S-KT) in the elderly patients following 50 mg doses. However, with an increase in dose and/or a decrease in renal function this increased concentration of conjugates may reach a point where the availability of uridyldiphosphate (UDP) glucuronyl transferase system becomes the rate limiting step. Consequently, this results in the saturation of the drug elimination through formation of conjugates. The t½ may be further prolonged, if following the accumulation caused by reduced renal function, the conjugated KT is hydrolysed *in vivo* back to the parent drug.[14] Despite the presence of considerable quantities of conjugated KT enantiomers (Table 4-6,4-7

and Fig. 4-2) the t½ of the unchanged KT enantiomers in the elderly patients reported here was not significantly affected due, perhaps, to the 1) smaller doses that were given in this study, and 2) the renal functions of our patients were not greatly impaired (creatinine clearance, 51 ±9.4 mL/min). Advenier *et al.*[13] also reported that their patients had normal serum creatinine for their age and sex. However, the average age of their patients was considerably more than ours (86.3±2.4 vs 72±4.6 yr). Stafanger *et al.*[15] noted a significant elevation of KT t½ in patients with renal dysfunction where the reported mean creatinine clearance was 37 mL/min.

An alternative explanation for the discrepancy between our observation and that of Advenier *et al.*[13] may be that the hydrolysis of conjugated KT back to the parent compound may occur *in vitro* resulting in overestimation of the pharmacokinetic parameters of intact KT in young *versus* elderly patients. This *in vitro* hydrolysis during analytical procedures has been previously noted.[16,17]

The observed ratio of conjugated S<R KT excreted in urine, was virtually identical to that reported previously. Collection of conjugated R- and S-KT in urine indicated a statistically greater amount of S-KT excreted after administration of the racemic dose. The cumulative amounts of both enantiomers did not support bioinversion of R- to S-KT, as the total amount of conjugated S-KT recovered did not exceed 25 mg (i.e., 50% of the racemic dose). On the whole, a trend existed whereby smaller amounts of the conjugated drug were excreted in elderly patients. The difference, however, was not statistically significant due to the unexpectedly low urinary

excretion of conjugated KT in patient 2 after multiple doses (10.26 mg, Table 4-3). This may have resulted from inadvertent loss of some portion of urine, as following a single dose the same patient excreted a total of 38.24 mg of conjugated KT. The reduced urinary excretion of KT conjugates in the elderly became significant when patient 2 was deleted from Table 4-3. Although, a reduced urinary excretion might imply a reduced absorption, Delbarre *et al.*[18] previously reported complete bioavailability for KT in the elderly. In the elderly, an age induced decreased renal function may cause enhanced biliary excretion of the conjugated drug.[19] Nevertheless, the S to R ratio of conjugated drug excreted in urine, did not change with advanced age. Despite the greater cumulative amount of conjugated S-KT in urine of all patients, the $Cl_r$ was greater for the R-enantiomer, as determined from the elderly patient data. The $Cl_r$ was not calculated for young patients, as plasma concentrations of conjugated KT enantiomers were negligible. Similarly, data generated from patients' 16 and 17, and from 15 and 16 after single and multiple doses, respectively, were not used in statistical determinations as no conjugated R-KT was detectable in the plasma of these patients. This aforementioned absence of conjugated R-KT in some patients' plasma, in fact, reinforced the finding that $Cl_r$ was greater for conjugated R-enantiomer.

Our data indicated substantial presence of conjugated S-KT in plasma of elderly patients which was preferentially eliminated in urine (i.e. S:R ratio in urine >1). An explanation for this observation may be that although conjugated S-KT is eliminated to a greater extent in urine, its elimination may be impaired when renal

function is reduced. However, in such a patient, R-KT may be more readily eliminated from the body by its elimination through bile. Upon increase in dose or further reduction in the renal function the formation of the conjugates may become the rate limiting step causing accumulation of the unchanged drug.

## 4.5. References


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# 5. PHARMACOKINETICS OF KETOPROFEN ENANTIOMERS IN CHOLECYSTECTOMY PATIENTS: INFLUENCE OF PROBENECID*

## 5.1. INTRODUCTION

Ketoprofen (KT, ±2-(3-benzoylphenyl) propionic acid), a 2-arylpropionic acid (2-APA) derivative is a non-steroidal antiinflammatory drug (NSAID) which is manufactured and administered as a racemic mixture. Recently, we have reported[1,2] that the pharmacokinetics of KT enantiomers exhibit only limited stereoselectivity in plasma of humans. Concentrations of conjugated S-KT in plasma, however, are greater than those of its antipode in elderly patients.[2] Conjugated S-KT was also predominant in urine. In healthy subjects, only a negligible amount of intact KT was found in urine, whereas an average of approximately 80% of the administered dose was found as conjugated KT.

In elderly patients,[2] and in those receiving probenecid (PB),[3] the urinary excretion of conjugated KT is reduced and the eventual elimination of total drug is not clearly understood. It has been suggested[1,2] that biliary excretion of KT may, in part, contribute to the overall drug clearance, however this has not been

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* A version of this chapter has been submitted for publication: R.T. Foster, F. Jamali, and A.S. Russell. Eur. J. Clin. Pharmacol., 1988.

documented for man. Moreover, a compensatory mechanism may exist between renal and biliary elimination.[4] Additionally, although it is known that enterohepatic recirculation of KT is significant in rats,[5] its possible contribution and significance in man is unknown.

It has been shown for 2-APA NSAIDs such as ibuprofen[6,7] and fenoprofen[8] in man, and KT in rat[5] that the R enantiomers undergo a unidirectional metabolic inversion to the S enantiomer. Presence of such a metabolic pathway can be proven unequivocally by administering the R enantiomer and detecting its antipode. Alternatively, in cases where the pure enantiomers are not available, recovery of greater than 50% of the administered racemic dose as the S enantiomer proves inversion. For drugs such as KT that undergo acyl glucuronidation, the biliary route may be an elimination pathway.[5] Hence, collection of bile and urine may allow for almost complete recovery of the administered racemic dose and provide neccesary information regarding enantiomer inversion.

To gain further insight into the pharmacokinetics of KT enantiomers in man, racemic doses were administered to cholecystectomy patients to 1) assess the contribution of the biliary route to the overall elimination of drug, 2) examine the possibility of the KT enantiomer inversion and, 3) study the influence of PB on the pharmacokinetics of KT enantiomers.

## 5.2. METHODS

### 5.2.1. Drug administration and sample collection

The project was conducted in accordance with the principles of the Declaration of Helsinki. Five patients (Table 5-1) who were to undergo a cholecystectomy, with subsequent T-tube drainage of bile, volunteered to participate in the study.

Following a four day recovery period from surgery, each patient received a 50 mg capsule of racemic ketoprofen (Rhone Poulenc Pharma. Inc., Montreal, Canada) at 0800 h, with one glass (200 mL) of water, following an overnight fast. After drug administration, venous blood (4-5 mL) was collected into heparinized tubes via an indwelling catheter inserted into a forearm vein. Blood samples were collected at 0, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 6, 8 and 12 h. Total urine output was collected at 0, 3, 6, 12 and 24 h. Similarly, total bile output was collected at 0, 2, 4, 6, 8, 10, 12 and 24 h. On a subsequent day, 1 g of PB (MSD, Kirkland, Canada) was administered 1.5 h prior to a 50 mg capsule of racemic KT. Blood, urine and bile were again collected in a fashion similar to that previously described, with the exception that both urine and bile were collected over additional intervals of 36 and 48 h to ensure complete recovery of drug. All specimens were stored in nitric acid rinsed containers at -20° prior to analysis. No other NSAIDs were allowed during, or two days prior to the study.

### 5.2.2. Determination of drug and conjugates

A previously reported stereospecific HPLC assay was utilized to analyze intact, and KT enantiomers as conjugates in the specimens.[9]

Table 5-1. Patient Characteristics

| Patient | Age, years | Sex | Weight kg | Creatinine Clearance mL/min |
|---|---|---|---|---|
| 1 | 56 | M | 96 | 86.2 |
| 2 | 70 | M | 48 | 46.7 |
| 3 | 54 | M | 79 | 56.8 |
| 4 | 41 | F | 51 | 71.6 |
| 5 | 58 | F | 66 | 86.0 |
| Mean | 56 | | 68 | 69.5 |
| SD | 10 | | 20 | 17.6 |

Briefly, after acidification of the specimen and extraction of KT enantiomers with 5% isopropanol/isooctane the organic layer was separated and 3 mL water added. The aqueous layer was separated and acidified. After addition of 3 mL chloroform the solution was Vortex-mixed and the aqueous layer discarded. Chloroform was evaporated and the residue derivatized with L-leucinamide in the presence of ethyl chloroformate. After addition of water, the solution was injected into an HPLC equipped with a UV detector (at 275 nm). The mobile phase (36% acetonitrile: 64% 0.06 M potassium phosphate: 0.02% triethylamine) was pumped with a flow of 1 mL/min through an end-capped, 10 cm, stainless steel column containing 5 micron reversed-phase packing material.

When analyzing bile, 2N perchloric acid (1:1 with bile), was utilized prior to extraction for sample clean-up. The subsequent extraction and derivatization steps were the same as previously reported.[9] Glucuroconjugated KT was stable in the presence of this acid.

Plasma, urinary and biliary concentrations of individual enantiomers were measured before and after alkaline hydrolysis. The concentrations of the ester conjugates were measured as the difference between the hydrolyzed and unhydrolyzed samples and are expressed based on equivalent intact KT.

5.2.3. Treatment of Data

Plasma enantiomer concentrations were plotted *versus* time. The area under the plasma KT concentration-time curve (AUC) was calculated utilizing the linear trapezoidal rule. The terminal

elimination rate constant ($\beta$), was determined from the best-fit line through the log-linear terminal phase of the curves. The plasma clearance (Cl/F = dose/AUC) and volume of distribution (Vd = Cl/$\beta$) were calculated for R-KT where the dose was considered to be ½ of the administered dose (25 mg). These calculations were not made for S-KT, as the possibility of R- to S-isomer inversion could not be ruled out, and hence the fraction of the dose, F, entering the systemic circulation as S-KT remained unknown.[1,2] Half-life ($t_{½}$) was calculated as 0.693/$\beta$. Differences in the pharmacokinetics between S- and R-KT, and the influence of PB were tested for significance using the Student's *t-test* for paired data ($\alpha$ = 0.05). The data are expressed as mean±SD.

## 5.3. RESULTS AND DISCUSSION

### 5.3.1. Ketoprofen alone

Figure 5-1a depicts the plasma KT enantiomer concentrations *versus* time profile in patient 1 as a representative of the patient population. Table 5-2 summarizes the pharmacokinetic parameters in all patients.

Plasma KT concentrations tend to be stereoselective in all but one patient (patient 2). During the post-absorptive phase, concentrations of S-KT were significantly greater than those of R-KT in patients 1, 3 and 5. Patient 4, on the other hand, had greater concentrations of R- compared to S-KT. Previous studies on healthy subjects,[1] and young and elderly arthritic patients[2] also indicated little or no stereoselectivity in the plasma concentration patterns of KT. In these cholecystectomy patients, however, the

Table 5-2. Pharmacokinetic Parameters in Patients after a 50 mg Dose of Racemic Ketoprofen

| Patient | 1 | 2 | 3 | 4 | 5 | Mean (SD) |
|---|---|---|---|---|---|---|
| **PLASMA** | | | | | | |
| $T_{max}$, h | | | | | | |
| S | 4.0 | 1.0 | 1.0 | 0.5 | 1.5 | 1.6 (1.4) |
| R | 4.0 | 1.0 | 1.0 | 0.5 | 1.5 | 1.6 (1.4) |
| $C_{max}$, mg/L | | | | | | |
| S | 1.09 | 2.12 | 2.41 | 5.11 | 2.14 | 2.57 (1.50) |
| R | 1.00 | 2.36 | 2.31 | 5.19 | 2.06 | 2.58 (1.56) |
| t½, h | | | | | | |
| S | 2.47 | 2.08 | 1.89 | 0.85 | 1.75 | 1.81 (0.60) |
| R | 2.47 | 2.00 | 1.61 | 1.33 | 1.69 | 1.82 (0.43) |
| AUC, (mg/L)h | | | | | | |
| S | 4.74 | 7.94 | 5.64 | 4.78 | 5.04 | 5.63 (1.34) |
| R | 4.22 | 8.65 | 4.85 | 5.56 | 4.37 | 5.53 (1.82) |
| Vd/F, L | | | | | | |
| R | 21.1 | 8.34 | 12.0 | 8.66 | 13.9 | 12.8 (5.19) |
| Cl/F, L/h | | | | | | |
| R | 5.92 | 2.89 | 5.15 | 4.50 | 5.72 | 4.84 (1.22) |
| $\Sigma Xu_{0-24}$, mg | | | | | | |
| S | 21.1 | 11.4 | 13.3 | 16.0 | 18.4 | 16.0 (3.87) |
| R | 12.8 | 8.57 | 8.86 | 10.9 | 12.2 | 10.7 (1.89)[a] |
| **BILE** | | | | | | |
| $C_{max}$, mg/L | | | | | | |
| S | 1.61 | 4.04 | 0 | 2.66 | 3.66 | 2.39 (1.64) |
| R | 2.18 | 2.60 | 0 | 2.15 | 3.16 | 2.02 (1.20) |
| $\Sigma Xb_{0-24}$, mg | | | | | | |
| S | 0.31 | 0.41 | 0 | 0.10 | 0.49 | 0.26 (0.21) |
| R | 0.37 | 0.27 | 0 | 0.07 | 0.30 | 0.20 (0.16) |

[a]Significantly different from the S-enantiomer.

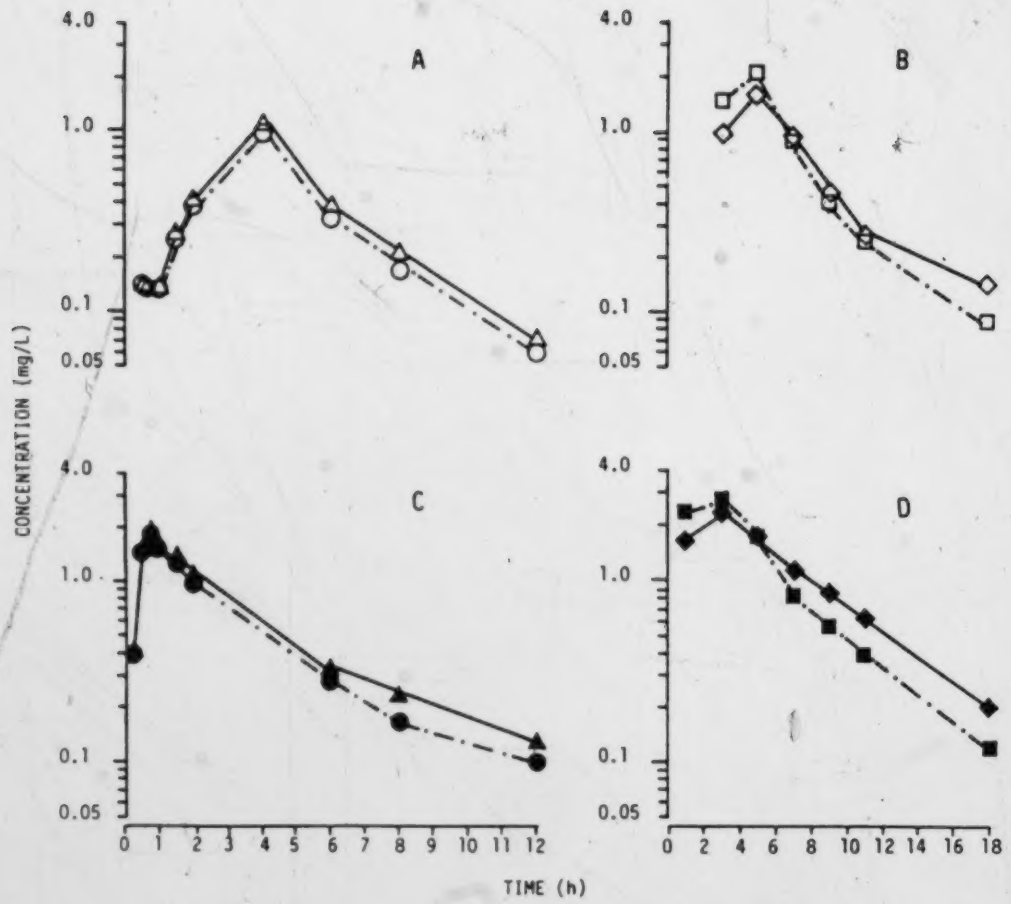


Fig.5-1. Ketoprofen concentration *versus* time curves in plasma (S = △ ; R = ○) and bile (S = ◇; R = □). Figures (A) and (B) depict plasma and bile, respectively, after a single 50 mg racemic oral dose of ketoprofen in patient 1. Figures (C) and (D) depict plasma and bile curves, respectively, after administration of 1000 mg probenecid prior to ketoprofen in the same patient.

small differences between the two enantiomers concentrations tended to increase with time and were greater (maximum AUC S:R ratio 1.16) than those observed previously in intact patients (maximum AUC S:R ratio 0.99). Despite the observed trend of stereoselectivity, differences between AUCs and t½s of R- and S-KT, however, were insignificant due to the observed inter-subject variation.

The mean t½ values for both enantiomers were shorter (S, 1.81; R, 1.82 h) than those previously reported by us for healthy subjects (S, 2.51; R, 3.80 h).[1] However, a smaller mean Vd/F in these patients (12.8 vs 19.1[1] L for R) was accompanied by a shorter mean t½ and resulted in similar AUCs between the two groups of subjects. Shorter t½s in cholecystectomy patients may be attributed to the constant drainage of bile, and hence, the excretion of conjugated drug. This interruption of enterohepatic recirculation of intact drug has previously been reported for KT enantiomers in bile-duct cannulated rats.[5] In humans, however, the contribution of such a mechanism appears negligible for KT as the cumulative biliary excretion of both conjugates did not exceed 2% of the administered dose. Alternatively, the difference in the pharmacokinetics of KT enantiomers in these patients as compared to healthy individuals may be explained by virtue of the patients' post-surgical condition alone.[10-12]

Only negligible concentrations of conjugated KT enantiomers were found in plasma. Using a nonstereospecific assay, Upton *et al.*[3] reported that maximal concentrations of conjugates were in the order of 10% of those for parent drug. This corresponds to approximately 5% for each enantiomer which is within the experimental

error of our stereospecific assay.

Bile samples contained negligible amounts of unchanged KT. Despite high biliary concentrations (up to 4.04 mg/L of S-KT and 3.16 mg/L of R-KT), the conjugated KT enantiomers in bile constituted only a small fraction of the dose (up to 1.96% of S-KT and 1.48% of R-KT). The observed biliary elimination of KT, however, is probably underestimated as a result of 1) T-tube drainage of bile does not allow for 100% recovery of bile[11,13] and, 2) bile flow in man is depressed for one to two weeks after surgery[12] and biliary excretion may be flow dependent. Nevertheless, enterohepatic recirculation is not likely to contribute significantly to the pharmacokinetics of KT enantiomers.

The biliary concentration of conjugated S-KT was consistently greater than that of conjugated R-KT except for some initial samples (Fig 5-1b). The concentrations of conjugated enantiomers in bile peaked within 3 to 5 h and declined with t½s ranging from 1.73 to 5.67 h. The biliary excretion rate *versus* time curves were parallel to the bile concentration *versus* time curves. This indicated that fluctuations in bile flow over the collection period were negligible, as suggested by others.[12] Moreover, the slope of the terminal log-linear portion of the curves may, in fact, reflect either the process of conjugation (formation dependent) or the biliary excretion of the formed conjugates (elimination dependent).

As concentrations of conjugated KT were greater in bile than in plasma, one may conclude that the passage of conjugated drug into bile is an active process, similar to that suggested for indomethacin.[13] Alternate suggestions such as uptake of

conjugated KT into biliary micelles could also explain the observed high bile/plasma concentration ratios. However, as KT glucuronides have a relatively high molecular weight and are more polar compared to intact drug, the former suggestion seems the likeliest.[4]

Negligible amounts of intact KT enantiomers were eliminated in urine. The cumulative urinary elimination of conjugated KT was stereoselective as consistently greater amounts of conjugated S-KT (16.0±3.87 mg) were eliminated compared to the antipode (10.7±1.89 mg). The total urinary recovery, however, was generally at the lower end of the previously reported range for healthy subjects.[1] This could be attributed to the observed lower creatinine clearance of these patients (46.7 - 86.2 mL/min) as compared to those subjects in the previous study (84 - 142 mL/min).[1] Interestingly, positive linear relationships were observed between the cumulative urinary excretion of conjugated KT enantiomers and creatinine clearance ($r^2$ = 0.94 for S and 0.97 for R) (Fig. 5-2). A relationship between total urinary excretion of all diflunisal species and creatinine clearance has similarly been observed.[14]

Although total output of both bile and urine were collected and the unchanged and conjugated KT concentrations were measured, only an average of approximately 54% of the administered dose could be accounted for (Table 5-2). Consequently, one may suggest a reduced absorption in this group of patients. The comparable AUCs, however, between patients in the present study and those previously reported for healthy subjects,[1] may dispute the possibility of reduced absorption. Alternatively, the presence of another elimination pathway (eg. hydroxylation) might be another explanation. This

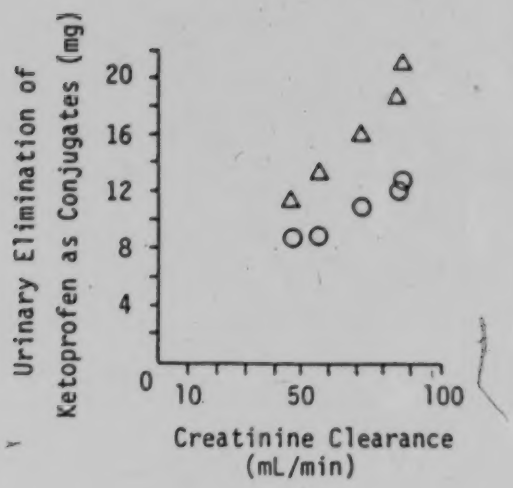


Fig. 5-2 Cumulative urinary elimination of ketoprofen (S=△; R=○) *versus* creatinine clearance in patients after oral administration of 50 mg racemic ketoprofen.

pathway of metabolism has previously been reported for rats.[15] However, we were unable to detect other metabolites due to the unavailability of authentic standards.

In this situation, the incomplete urinary and biliary recovery of KT does not permit definitive conclusions regarding enantiomeric inversion of R- to S-KT. However, for other 2-APA NSAIDs, inversion has been proven in man.[6-8]

5.3.2. Ketoprofen and Probenecid

Probenecid did not influence the pattern of stereoselectivity in the pharmacokinetics of KT (Fig. 5-1c, table 5-3). However, PB coadministration significantly prolonged t½ and reduced Cl/F of both KT enantiomers. Consequently, AUC values for both R- and S-KT were significantly elevated. These findings were in agreement with those previously reported by Upton *et al.*[3] who further found a significant 4- to 5-fold increase in the plasma concentration of conjugated KT following coadministration of PB given as 500 mg/6 h. However, we found no significant rise in the observed negligible concentrations of the conjugated KT in plasma as a result of PB treatment. One may attribute this discrepancy to *in vitro* hydrolysis of conjugates as previously reported for other glucuroconjugated compounds.[16,17] However, our collection and storage procedures were designed to minimize hydrolysis of conjugated KT. Moreover, we analyzed several samples immediately after collection to minimize hydrolysis of the ester conjugates and found only insignificant concentrations of conjugates. Furthermore, utilizing this assay, we previously observed considerable

Table 5-3. Pharmacokinetic Parameters in Patients after 50 mg Racemic Ketoprofen and 1 g Probenecid

| Patient | 1 | 2 | 3 | 4 | 5 | Mean (SD) |
|---|---|---|---|---|---|---|
| PLASMA | | | | | | |
| $T_{max}$, h | | | | | | |
| S | 0.75 | 0.25 | 1.0 | 1.0 | 2.0 | 1.0 (0.64) |
| R | 0.75 | 0.25 | 1.0 | 1.0 | 2.0 | 1.0 (0.64) |
| $C_{max}$, mg/L | | | | | | |
| S | 1.91 | 7.98 | 2.49 | 5.14 | 2.70 | 4.04 (2.52) |
| R | 1.81 | 7.83 | 2.31 | 5.78 | 2.52 | 4.05 (2.63) |
| t½, h | | | | | | |
| S | 4.52 | 5.39 | 3.95 | 1.57 | 3.66 | 3.82 (1.42)[b] |
| R | 4.17 | 3.78 | 2.35 | 1.51 | 2.76 | 2.91 (1.08)[a,b] |
| AUC, (mg/L)h | | | | | | |
| S | 7.45 | 32.0 | 10.3 | 9.55 | 12.0 | 14.3 (10.1)[b] |
| R | 6.46 | 23.8 | 6.65 | 11.6 | 8.05 | 11.3 (7.27)[b] |
| Vd/F, L | | | | | | |
| R | 23.3 | 5.72 | 12.8 | 4.72 | 12.4 | 11.8 (7.43) |
| Cl/F, L/h | | | | | | |
| R | 3.87 | 1.05 | 3.76 | 2.16 | 3.11 | 2.79 (1.19)[b] |
| $\Sigma Xu_{0-48}$, mg | | | | | | |
| S | 21.3 | 6.23 | 7.10 | 8.35 | 11.1 | 10.8 (6.16)[b] |
| R | 9.92 | 1.89 | 2.45 | 3.91 | 3.35 | 4.30 (3.24)[a,b] |
| BILE | | | | | | |
| $C_{max}$, mg/L | | | | | | |
| S | 2.45 | 6.51 | 0.43 | 8.74 | 10.4 | 5.70 (4.19) |
| R | 2.88 | 3.07 | 0.20 | 5.21 | 6.96 | 3.66 (2.56) |
| $\Sigma Xb_{0-48}$, mg | | | | | | |
| S | 0.61 | 1.43 | 0.03 | 0.70 | 0.70 | 0.69 (0.50) |
| R | 0.59 | 0.43 | 0.01 | 0.41 | 0.50 | 0.39 (0.22)[b] |

[a]Significantly different from the S-enantiomer.
[b]Significantly altered by PB co-administration.

concentrations of KT conjugates in elderly patients' plasma.[2] A more likely explanation involves the competitive reduction of glucuroconjugation of KT caused by the single, but larger, dose of PB[18] given in this study as compared to that in the study by Upton *et al.*[3] Furthermore, Sorgel *et al.*[19] suggested that PB may actually reduce the availability of uridinediphosphate glucuronyltransferase. These findings not only suggest reasons for the negligible presence of conjugated enantiomers in plasma, but also explain, at least in part, the reduced urinary elimination of conjugates as a consequence of their reduced formation.

The cumulative urinary elimination of both conjugated KT enantiomers was significantly reduced by PB administration. Elimination of intact KT was negligible. On a similar basis, PB has been shown to interact with drugs such as indomethacin,[20-22] naproxen,[23] carprofen,[24] zomepirac[25] and clofibrate.[26,27]

PB is also known to inhibit renal tubular secretion of organic acids.[4] In such cases where renal elimination is reduced, one might expect the biliary pathway to act as an alternative elimination route. This compensatory interrelationship between urinary and biliary elimination has previously been observed for other drugs when renal or hepatic function is compromised.[4,28,11,14] However, PB is also known to competively reduce biliary secretion of drugs from hepatocytes.[4] Nevertheless, when PB and KT were coadministered in the present study, an increase in the cumulative amounts of both R- and S-KT (49 and 59%, respectively) excreted in bile (fig. 5-1d) was observed. A trend, therefore, of greater conjugated S-KT in bile after PB administration was observed. Despite this trend, however, a

significantly increased cumulative biliary elimination was only observed for conjugated R-enantiomer and not the S-enantiomer. This finding was perhaps due to the inter-subject variability. The increased biliary elimination caused by PB, however, did not totally account for the reduced urinary excretion of KT conjugates. The unrecovered portion of the dose might have been eliminated through other metabolic routes as explained in the previous section. Findings of the present study coupled with those of previous studies of naproxen[23] and zomepirac[25] indicate that PB primarily suppresses phase II, rather than phase I metabolism.

This study indicates that biliary elimination of both R- and S-KT as conjugates is a minor elimination pathway. Consequently, the extent of enterohepatic circulation of KT is negligible. Moreover, when PB and KT are administered concurrently, conjugation and subsequent renal elimination of conjugated KT is impaired. In partial compensation for this, the biliary elimination of glucuroconjugated drug is enhanced. This, however, is not a total compensatory mechanism suggesting that perhaps phase I metabolism has also been enhanced. Furthermore, it was observed that these patients, on average, exhibited different pharmacokinetics than previously reported.[1,2] These findings may be attributed to the very nature of the patients' conditions.

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## 6. Ketoprofen Enantiomers in Synovial Fluid*

### 6.1. INTRODUCTION

Over the past few years, there has been widespread interest in the implications of stereochemistry on the pharmacokinetics of racemic drugs.[1-5] This interest, in part, stems from the realization that individual drug enantiomers may exhibit different pharmacokinetics.

An important group of chiral compounds, the 2-arylpropionic acid (2-APA) non-steroidal antiinflammatory drugs (NSAIDs), are usually administered as racemates. The two enantiomers not only differ in their antiinflammatory properties, but with few known exceptions,[6-8] also have different pharmacokinetics.[4-5] Nevertheless, we are still witnessing the appearance of research articles describing the time-course of these NSAIDs with no consideration given to the fact that data generated by non-stereospecific assays can be misleading.[1,2]

The NSAIDs are primarily used in the treatment of joint disorders, thus, it is proposed that the site of their action is the synovium.[9,10] Therefore, in studying the antiinflammatory efficacy/plasma concentration relationship, an understanding of the time course of the drug in synovial fluid is essential.[9] Recently

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Netter *et al.* [11] have reported the total and protein-free concentration of ketoprofen in serum and synovial fluid. Unfortunately, however, as their data does not differentiate between the enantiomers, the contribution of the therapeutically active S-enantiomer remains unknown.

## 6.2. METHODS

### 6.2.1. Drug administration and sample collection

The project was conducted in accordance with the principles of the Declaration of Helsinki.

In this communication we report our recent observations regarding the steady-state plasma and synovial fluid concentrations of ketoprofen enantiomers in six rheumatoid- or osteo-arthritic patients who were under therapy with ketoprofen for at least 3 days and required knee aspiration (Table 6-1).

Depending on the attending physician's assessment of each patient, the administered doses ranged from 150 to 200 mg daily, given as 50 mg capsules (Rhone-Poulenc Pharma Inc, Montreal, Canada) and in various regimens (Table 6-1). During the study, and two weeks prior, no other NSAIDs were administered. At the time the knee aspiration was performed, a blood sample (4-5 mL venous blood) was collected into a heparinized tube. Plasma was separated by centrifugation, and both synovial fluid and plasma samples were

Table 6-1. Steady-State Concentration of Ketoprofen Enantiomers in Plasma and Synovial Fluid

| Patient | Dose, mg | Sampling Time h,post-dosing | Synovial Fluid mg/L | | | Plasma mg/L | | | Synovial Fluid/Plasma | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | S | R | S/R | S | R | S/R | S | R |
| 1 | 50 tid | 2.5 | 0.43 | 0.43 | 1.00 | 1.09 | 1.04 | 1.05 | 0.40 | 0.41 |
| 2 | 100 bid | 5.0 | 0.91 | 0.80 | 1.14 | 1.39 | 1.18 | 1.18 | 0.66 | 0.68 |
| 3 | 100 bid | 15.0 | 0.14 | 0.14 | 1.00 | 0.05 | 0.04 | 1.25 | 2.93 | 3.64 |
| 4 | 50 qid | 8.0 | 0.47 | 0.45 | 1.04 | 0.38 | 0.33 | 1.15 | 1.24 | 1.37 |
| 5 | 50 qid | 8.0 | 0.25 | 0.24 | 1.04 | 0.18 | 0.16 | 1.13 | 1.43 | 1.52 |
| 6 | 50 qid | 4.0 | 0.42 | 0.42 | 1.00 | 2.41 | 2.29 | 1.05 | 0.17 | 0.18 |

stored at -20° until the time of analysis. A previously reported stereospecific HPLC method was used to analyze ketoprofen enantiomers in the specimens.[12]

### 6.2.2. Treatment of Data

Differences in the concentrations between S- and R-KT (in both plasma and synovial fluid), and differences in the synovial fluid/plasma concentrations for both enantiomers were tested for significance using the Student's *t-test* for paired data ($\alpha$ = 0.05).

## 6.3. RESULTS AND DISCUSSION

We have recently reported pharmacokinetics of ketoprofen enantiomers in plasma and urine of healthy individuals,[7] and in young and elderly arthritic patients:[8] the time courses of the individual enantiomers in plasma were, for the most part, very similar. On the other hand, the elimination of the conjugated ketoprofen in urine was stereoselective, the S-enantiomer being predominant. There was also substantial stereoselectivity with respect to the presence of conjugated ketoprofen in the plasma of elderly patients (S>R).[8] As the synovium is considered to be a part of an extravascular compartment,[9] the similarity observed in the time courses of the enantiomers in plasma may not be extrapolated to the synovial fluid.

Based on the data depicted in Table 6-1, one can conclude that the concentrations of the enantiomers in both plasma and synovial

fluid are not significantly different from one another.

A comparison of ketoprofen enantiomer concentrations in synovial fluid with those in plasma reveals an increasing ratio (synovial fluid/plasma) as time progresses through the measured period of time. This report is in line with others,[9-11] and indicates that, like many other NSAIDs, there is a delayed entry of drug into the synovial fluid. More importantly, for ketoprofen, there appears to be no difference between the rates of appearance of the enantiomers in synovial fluid.

The dissimilarity between the NSAIDs time courses in plasma and synovial fluid may explain, at least in part, the frequently observed poor efficacy/plasma concentration correlation.[10] A better correlation may, however, exist between the drug concentrations in plasma and synovial fluid during the post-distribution phase.

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## 7. Stereoselective Pharmacokinetics of Ketoprofen in the Rat: Influence of Route of Administration*

### 7.1. Introduction

The non-steroidal antiinflammatory drugs (NSAIDs) are of great clinical importance in the treatment of rheumatoid and osteoarthritis. An important sub-group of NSAIDs, the 2-arylpropionic acid (2-APA) derivatives, such as ketoprofen (KT), possess a chiral center, and exist as racemates. Although the literature contains numerous scientific articles dealing with the clinical pharmacokinetics of these drugs[1] only recently have reports addressed the clinical significance of their stereoselective disposition.[2,3]

It has been observed that the antiinflammatory activities of the 2-APAs are ascribed to the S-isomer, while the R-enantiomers are either inactive or have reduced activity.[2] Upon administration of some 2-APAs, the R-isomers are inverted to the S-antipodes. The exact mechanism of inversion is not completely clear, although it appears to be mediated, in part, by coenzyme A.[2,4-6] More recently, it has been suggested that a significant portion of inversion may take place in the gastrointestinal tract during absorption.[7,8] Upon administration of KT to man, however, virtually no stereoselectivity in plasma concentrations of unchanged

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KT was observed. On the other hand, the urinary excretion of conjugated KT was stereoselective.[9,10] In elderly patient's plasma, however, a substantially greater amount of conjugated S-KT, as compared to R-KT, was found.[10]

Administration of KT to rabbit[11] resulted in significant stereoselectivity which was, in part, attributed to inversion. The major reason for the observed stereoselectivity, however, was attributed to differences in clearance of the enantiomers. Exploratory studies conducted by us, in rats, revealed substantial stereoselectivity in the disposition of KT enantiomers. The rat was used as a model to study the process of inversion despite differences between this species and man. It is our hypothesis that KT undergoes inversion in the rat, and that the gastrointestinal tract substantially contributes to this.

## 7.2. Materials and Methods

### 7.2.1. Chemicals

Ketoprofen was a gift from Rhone Poulenc Pharma. Inc. (Montreal, Canada). The internal standard, fenoprofen calcium, was supplied by Eli Lilly (Toronto, Canada). All other chemicals were HPLC grade.

### 7.2.2. Surgery

Male Sprague-Dawley rats weighing between 250-350 g were used for the study after approval was obtained from the Institutional Animal Welfare Committee. A total of 18 rats were catheterized with

silastic tubing (0.025 in. ID X 0.047 in. OD) at the jugular vein (Dow Corning, Michigan, USA). In addition, four rats were catheterized with polyethylene tubing (Clay Adams, New Jersey, USA) at the common bile duct, as well as at the jugular vein. During surgery, all animals were anesthetized with methoxyflurane (Pitman-Moore Ltd. Mississauga, Canada). The animals were allowed to recover overnight before the experiment, during which time they were individually housed in plastic metabolic cages. Animals that were bile duct cannulated were housed in restraining cages. During the time the experiment was conducted, the animals were given food and water *ad libitum*.

7.2.3. Dosing and Sample Collection

Racemic ketoprofen dissolved in polyethylene glycol 400 was administered (10 mg/kg) via the iv (*via* jugular vein cannula), ip and po routes. Blood (0.2 mL) was collected from the jugular vein cannula at 0, 0.25, 0.50, 1.0, 2.0, 3.0, 4.0, 6.0, 12.0, and 24.0 hours after administration. Between each blood sample collection, the catheter was heparinized (10 u/mL). Urine was collected at intervals of 0-3, 3-6, 6-9, 9-12, 12-24, 24-48, and 48-72 hours after drug administration. Bile was collected at intervals of 0-3, 3-6, and 6-9 hours after drug administration. Blood was centrifuged and the plasma portion separated. All samples were immediately frozen at -20° until analyzed.

7.2.4. Assay

Concentrations of KT in plasma and amounts of conjugated KT in

urine and bile were determined by a previously reported stereospecific HPLC method.[12] The amount of conjugated KT eliminated in bile and urine was estimated following alkaline hydrolysis.[12] The difference in amount of drug before and after this hydrolysis was taken as the contribution of the conjugated KT.

7.2.5. Pharmacokinetic Analysis

The area under the plasma concentration-time curve (AUC) was calculated by the linear trapezoidal rule. The area from the last concentration point ($C_{last}$) to infinity was calculated as $C_{last}/\beta$, where $\beta$ is the terminal elimination rate constant. In some instances, however, the true $\beta$ could not be estimated following po doses. This was due either to very low concentrations of R-KT or fluctuations in plasma concentrations of S-KT. Alternatively, in these cases, the mean value of $\beta$ obtained after iv and ip dosing was used in calculations (Table 7-I). The fraction inverted following iv doses was calculated as ΣXS-Dose/Dose, where ΣXS is the cumulative amount of recovery in bile as S-antipode. This is, however, an underestimation of the extent of inversion because the total recovery in bile-duct cannulated rats was less than 100% (Table 7-1). After po doses, on the other hand, the fraction inverted was calculated as $1-(AUC_R(po)/AUC_R(iv))$ — assuming that absorption was complete, and that inversion was the major pathway of presystemic elimination for R-KT.

7.2.6. Statistical Analysis

Differences between the enantiomer concentrations observed in

Table 7-1. Pharmacokinetic Indices After Single 10 mg/kg Racemic Doses of Ketoprofen

| Route of Administration | AUC, (mg/L)h | | | t½, h | | Vd, L | | %dose in bile | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | S | R | S/R | S | R | S | R | S | R | S/R |
| *iv* | | | | | | | | | | |
| (bile-duct cannulated) | 170 | 50.2 | 3.39 | 1.1 | 0.46 | 0.03 | 0.02 | 80.6 | 11.7 | 6.89 |
| | 62.5 | 13.3 | 4.70 | 0.95 | 0.79 | 0.05 | 0.11 | 73.0 | 7.83 | 9.32 |
| | 36.0 | 11.0 | 3.28 | 0.67 | 0.55 | 0.07 | 0.16 | 81.1 | 13.4 | 6.03 |
| | 73.0 | 16.8 | 4.35 | 0.63 | 0.79 | 0.02 | 0.05 | 76.9 | 12.6 | 6.09 |
| MEAN | 85.4[a] | 22.8[a] | 3.93[a] | 0.83[a] | 0.65[a] | 0.04[a] | 0.09[a] | 77.9 | 11.4 | 7.08 |
| SD | 58.6 | 18.4 | 0.70 | 0.21 | 0.17 | 0.02 | 0.06 | 3.77 | 2.48 | 1.54 |
| *iv* | | | | | | | | %dose in urine | | |
| | | | | | | | | S | R | S/R |
| | 362 | 77.3 | 4.68 | 10.0 | 24.4 | 0.14 | 0.67 | 15 | 3.1 | 4.9 |
| | 518 | 98.4 | 5.26 | 15.3 | 28.0 | 0.25 | 1.0 | 2.4 | 0.97 | 2.4 |
| | 1362 | 47.0 | 29.0 | 12.2 | 6.19 | 0.04 | 0.36 | 12 | 1.4 | 8.1 |
| | 886 | 82.3 | 10.8 | 10.5 | 16.7 | 0.06 | 0.75 | 4.4 | 1.1 | 4.1 |
| | 928 | 97.4 | 9.52 | 13.9 | 23.1 | 0.07 | 0.67 | 14 | 1.5 | 9.6 |
| MEAN | 811 | 80.5 | 11.8 | 12.4 | 19.7 | 0.11 | 0.69 | 9.5 | 1.6 | 5.8 |
| SD | 391 | 20.9 | 9.93 | 2.63 | 8.57 | 0.09 | 0.23 | 5.8 | 0.84 | 3.0 |
| *po* | | | | | | | | | | |
| | 297 | 8.17 | 36.3 | 17.5 | b | 0.25 | b | 4.4 | 0.75 | 5.9 |
| | 474 | 9.31 | 50.9 | b | b | b | b | 1.6 | 0.68 | 2.4 |
| | 474 | 14.1 | 33.6 | b | b | b | b | 6.6 | 1.1 | 6.0 |
| | 386 | 17.2 | 22.4 | b | b | b | b | 9.7 | 1.3 | 7.6 |
| | 400 | 15.7 | 25.5 | 11.0 | b | 0.17 | b | 4.8 | 0.45 | 11 |
| MEAN | 406 | 12.9 | 33.7 | b | b | b | b | 5.4 | 0.85 | 6.5 |
| SD | 73.4 | 3.96 | 11.2 | b | b | b | b | 3.0 | 0.33 | 3.0 |
| *ip* | | | | | | | | | | |
| | 744 | 50.4 | 14.8 | 12.3 | 4.29 | 0.06 | 0.19 | 16 | 1.4 | 12 |
| | 892 | 102 | 8.70 | 12.8 | 37.4 | 0.06 | 1.0 | 10 | 1.2 | 9.1 |
| | 353 | 36.1 | 9.80 | 10.2 | 11.5 | 0.14 | 0.83 | 8.3 | 1.4 | 5.6 |
| | 371 | 34.8 | 10.7 | 9.41 | 15.6 | 0.14 | 1.3 | 4.9 | 0.6 | 8.3 |
| MEAN | 590 | 55.9 | 11.0 | 11.2 | 17.2 | 0.10 | 0.82 | 10 | 1.2 | 8.7 |
| SD | 270 | 35.0 | 2.64 | 1.63 | 14.3 | 0.05 | 0.45 | 4.8 | 0.40 | 2.5 |

Duncan's M.R. Test:

| | *iv* | *ip* | *po* |
|---|---|---|---|
| AUC, S: | ——— | ——— | — |
| R: | ——— | | — |
| AUC, S/R: | ——— | | — |
| ΣXu, S: | ——— | ——— | |
| R: | ——— | ——— | |

| | | *iv* | *ip* | *po* |
|---|---|---|---|---|
| t½, | S: | ——— | | b |
| | R: | ——— | | b |
| Vd, | S: | ——— | | b |
| | R: | ——— | | b |

a. Significantly different from *iv*; b. Not calculated due to insufficient data points; Connecting lines indicate insignificant differences.

each individual rat were assessed by a paired Student's *t-test*. Differences in pharmacokinetic parameters between routes of administration were examined using one-way ANOVA. Where significant differences were found, Duncan's Multiple Range test was used to compare the means. The level of significance was set at $\alpha = 0.05$. All results are expressed as mean$\pm$SD.

## 7.3. Results and Discussion

There were no significant differences in the total (S- plus R-KT) AUC values following all three modes of administration. The drug was rapidly absorbed (po and ip doses) and distributed soon after dosing, regardless of route of administration (Fig. 7-1). There were no significant differences between the enantiomers with respect to their t½. Both R- and S-KT were eliminated mainly through biliary excretion as the ester conjugates (Table 7-1). Only a minor fraction of the administered dose was eliminated through the kidneys as the conjugated drug (Table 7-1).

### 7.3.1. Intravenous Doses

A pseudoequilibrium was rapidly attained after iv doses (Fig. 7-1). The AUCs of S-KT were on average, approximately 11-fold greater than those for R-KT (Table 7-1). The difference in AUCs of the enantiomers could be attributed to 1) inversion of R- to S-KT, and/or 2) different distribution or elimination kinetics.

The cumulative amount of drug excreted in urine was 9.5$\pm$5.8 and 1.6$\pm$0.84% for conjugated S- and R-KT, respectively (Table 7-1). Despite the trend where greater amounts of S-enantiomer were found in

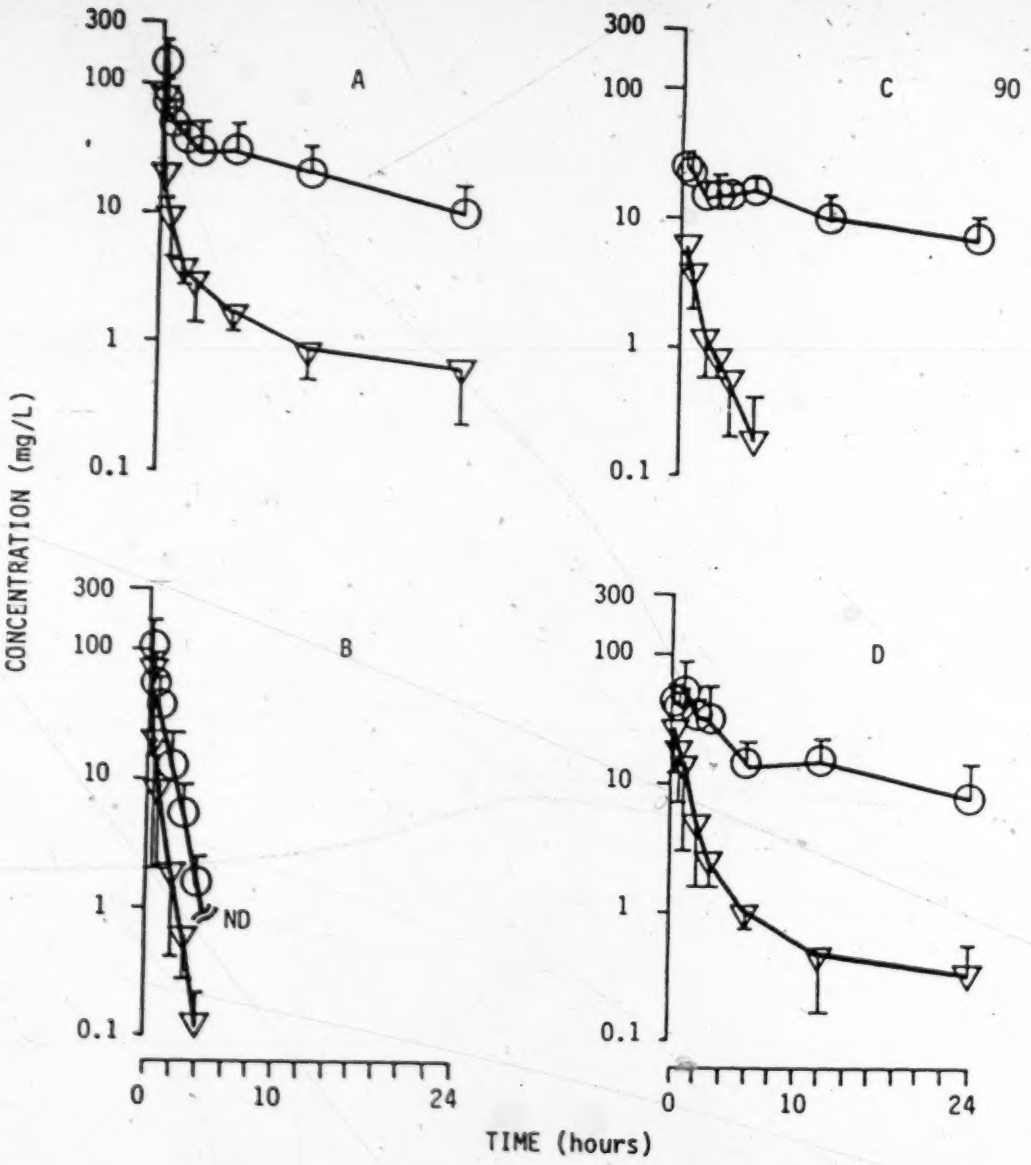


Fig. 7-1. KT concentration in plasma *versus* time profiles after, A) iv dosing, B) iv dosing to bile-duct cannulated rats, C) po dosing, and D) ip dosing. ND denotes unquantifiable concentrations at subsequent times. R-KT, ▽; S-KT, ○.

both plasma and urine, no conclusive data was generated for, or against, inversion. It does, however, indicate that the enantiomers of KT exhibit stereoselectivity in their disposition.

Proof of inversion can only be made by administration of pure R-KT, or by collection of >50% of the administered dose as S-configuration from the elimination pathways. As pure R-enantiomer was not readily available, and thus could not be used to prove bioinversion, four rats were bile duct-cannulated to enable complete collection of drug. Figure 7-1 depicts the mean plasma concentration versus time curve for bile duct-cannulated rats following iv doses. The results indicate that, similar to observations in intact rats, KT enantiomers demonstrated stereoselectivity. The AUCs, however, were significantly and substantially lower in the bile duct-cannulated rats when compared to intact rats dosed iv (Table 7-I). Similarly, the mean t½s of 0.83±0.21 and 0.65±0.17 h, were significantly shorter than 12.4±2.63 and 19.7±8.57 h for S- and R-KT in bile duct-cannulated and in intact rats, respectively. This indicates that enterohepatic recirculation must contribute substantially to the AUCs observed in intact rats.

Due, perhaps, to the constant drainage of bile, the volume of urine output was negligible. Consequently, urine was not collected from these animals. The mean cumulative amounts of S- and R-KT collected as conjugates in the bile duct-cannulated rats were 77.9±3.77 and 11.4±2.48% of the dose, respectively. This is indicative of at least a 56% R to S inversion. The results prove four important points that 1) unlike humans, rat biliary excretion is the major elimination pathway for KT, 2) there is substantial

enterohepatic recirculation, 3) as almost 78% of the given racemic dose was recovered as the S-isomer, there is significant R- to S-KT inversion in rats, and 4) in the bile duct-cannulated rats, inversion is not taking place during recirculation and subsequent reabsorption. (Table 7-I).

7.3.2. Oral Doses

In light of suggestions made by Simmonds *et al.*[7] for benoxaprofen, and by Jamali *et al.*[8] for ibuprofen, regarding the contribution of the gastrointestinal tract to inversion, five rats were dosed orally. Similar to iv doses, plasma concentrations of S-KT were significantly greater than R-KT (Fig. 7-1). However, the extent of the difference between the AUC of enantiomers was substantially greater after oral (S/R = 33.7) than after iv (S/R = 11.8) doses (Table 7-1). This was mainly due to the fact that significantly lower AUCs were observed for R-KT after po dosing compared to those after iv dosing ($12.9 \pm 3.96$ *vs* $80.5 \pm 20.9$). The AUCs of S-KT and S-plus R-KT, on the other hand, were not significantly different, although a trend of lower AUCs after po dosing as compared to iv dosing was noticed. The disproportional reduction in the AUCs of R-KT after oral dosing compared to those after iv doses might be attributed to stereoselective absorption and/or first-pass metabolism. The urinary data which revealed no significant differences between the three routes of administration could not confidently be used to assess bioavailability because, in all cases, the total urine output contained less than 11% of the dose as conjugated enantiomers (Table 7-1). Although the possibility of

stereoselective absorption cannot be ruled out, due to the passive and nonselective nature of the process,[13] it is less likely to be responsible for this observation. Thus, the significantly reduced AUC of R-KT after po, as compared to iv dosing, is likely due to a first-pass metabolism and possibly its inversion to S-KT. In the latter case one would expect a greater AUC for S-KT after oral doses. However, after iv dosing, the differences between the AUCs of the S-enantiomers and the inter-animal variation is so wide that they may conceal a further increase in the extent of inversion after oral doses. Nevertheless, the presystemic inversion for an oral dose of KT appears to be approximately 84% after po doses. This is assuming that inversion is the sole pathway of clearance of R-KT. Our data supports such a postulate, as the involvement of other metabolic pathways in the presystemic metabolism of R-KT, would likely result in smaller AUCs for S-KT; the reduction of S-KT AUCs being the result of lesser R- to S-inversion. However, as the differences between the AUCs of S-KT calculated following either iv, ip, or po doses were insignificant, one can rule out the possibility of the involvement of other major metabolic/excretory pathways.

7.3.3. Intraperitoneal Doses

To investigate the possibility of contribution of the gastrointestinal tract to the overall inversion, KT was administered via the ip route. If the gastrointestinal tract is a major site of inversion, data collected after this route of administration should be similar to that generated after iv dosing. Interestingly, after ip doses, the AUCs of S- and R-KT were similar to those observed

after iv dosing (Table 7-1). The S:R AUC ratios after ip dosing were 11.0±2.64 and very close to those after iv administration (11.8±9.93) and significantly less than that of 33.7±11.2 observed after oral dosing. In line with earlier reports dealing with benoxaprofen in rat[7] and ibuprofen in man,[8] this suggests that the gastrointestinal tract is, in part, reponsible for the presystemic enantiomeric inversion of KT in the rat. Nevertheless, for this drug, there is still a substantial amount of inversion following iv doses. According to recent reports, the kidneys, and to a lesser extent the liver, may in fact, be responsible for a large portion of the systemic inversion.[14]

In summary, the results of this work indicate that rat, in contrast to man and rabbit, is a very efficient invertor of R- to S-KT. The inversion appears to be largely a presystemic process, where at least 84% of the administered dose of R-KT is inverted to its antipode in the gastrointestinal tract. The observed S:R plasma concentration ratios support this suggestion. After all routes of administration, the S:R concentration ratio rose progressively with time. This rise, however, was not linear as it tended to be less pronounced after the first few samples were collected. This is analogous to the model previously suggested for a drug where both systemic and presystemic inversion contribute to the overall inversion process.[15] The substantial systemic inversion observed after both ip and iv dosing further support this suggestion.

Similar to ibuprofen,[16] the observed extensive enantiomeric inversion of KT in the rat appears to be consistent with its previously reported incorporation into adipose tissue.[17] This,

however, cannot be extrapolated to man in whom little or no inversion has been observed for KT.[9,10]

Biliary elimination of conjugated KT enantiomers accounts for approximately 87% of the administered dose, whereas urinary elimination is only approximately 11%. The previously reported predominant hydroxylation of KT in rats,[18] therefore, appears to be a minor pathway for KT elimination. In all cases, the majority of recovered drug is the conjugated S-isomer. Reabsorption of S-KT from the gastrointestinal tract after biliary excretion contributes to the longevity of this isomer.

## 7.4. References


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## 8. GENERAL DISCUSSION AND CONCLUSIONS

Prior to the present investigation it was thought that, in man, the 2-APA class of NSAIDs all displayed stereoselective pharmacokinetics, and that bioinversion of the lesser active R- to the more active S-enantiomer was a general phenomenon. Furthermore, numerous conclusions were also made in an attempt to extrapolate results collected from animal studies to therapy regarding humans. These generalities were postulated largely due to the fact that the 2-APA class of NSAIDs are a relatively homogeneous group of compounds with respect to their chemistry. To more thoroughly investigate individual members of the 2-APA class of NSAIDs KT was chosen as no data were available regarding its enantiospecific pharmacokinetics in animal or man.

The present study revealed some important, and perhaps suprising, findings. In healthy volunteers, it was demonstrated that 1) KT, unlike most 2-APA NSAIDs, did not exhibit stereoselective pharmacokinetics in plasma; in urine, however, there was a greater presence of conjugated S-KT, 2) inversion was minimal at most. In young and elderly arthritic patients, it was noted that 1) the pharmacokinetic parameters obtained from young arthritic patients were similar to those of healthy subjects, 2) elderly patients had significant concentrations of conjugated KT in plasma, 3) urinary elimination of KT as

conjugates was reduced in elderly arthritic patients, and 4) findings suggested biliary elimination of KT. The contribution of the biliary pathway to elimination of KT was subsequently investigated in another group of patients. This particular study unexpectedly revealed that KT was not significantly eliminated through bile. Moreover, in the presence of PB (an inhibitor of renal secretion) biliary elimination of KT did not completely compensate for the reduced urinary elimination of KT. Perhaps more importantly, it was suggested that other, otherwise minor pathways of elimination became more prominent, allowing for drug loss through other pathways (presumably phase I). As the recovery of total administered dose was incomplete, no firm conclusions regarding enantiomer inversion could be made. It appeared, however, that any contribution of inversion would be minimal at best.

As the clinical response of KT is primarily due to its activity in synovial fluid, it was necessary to investigate the disposition of the enantiomers in synovial fluid. A subsequent study revealed that the enantiomeric plasma concentration ratios of KT reflected those in synovial fluid.

This study also demonstrated the importance of species-dependent pharmacokinetics. Data collected from rats revealed that, unlike humans, 1) the pharmacokinetics of KT enantiomers was stereoselective, 2) there was R- to S-KT inversion, 3) urine was a minor elimination pathway of conjugated KT, and 4) the biliary

pathway of elimination was the major route of drug excretion. It was further reported that a substantial first pass inversion of R- to S-KT resulted upon passage of drug through the gastrointestinal tract. This study, therefore was able to pin-point the gastrointestinal tract as the major site of presystemic inversion, whereas the likely site of systemic inversion was the liver and/or kidneys. These results not only served to indicate important pharmacokinetic differences between man and rat, but further emphasized that data of this nature cannot be extrapolated to man.

For drugs such as KT, with limited or no enantiomeric inversion, the therapeutically inactive enantiomer may be considered as a substantial impurity of the dosage form. However, any attempts to delete the inactive enantiomer or adjust the enantiomeric composition of the dosage form must only be conducted after carefully considering the possibilities of interactions between the two enantiomers. Enantiomers may interact with one another, thereby altering pharmacokinetic parameters. In the absence of one enantiomer, therefore, the other may have a totally different pharmacokinetic profile.

As yet, with a few exceptions, a clear correlation between the antiinflammatory effects and plasma concentration of NSAIDs has not been reported. The data generated in this work, however, may provide a better understanding as to why such an unclear relationship exists.